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Anticonvulsant Activity of Δ^8- and Δ^9-Tetrahydrocannabinol in Rats[1]

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MCCAUGHRAN, J. A., JR., M. E. CORCORAN AND J. A. WADA. *Anticonvulsant activity of Δ^8- and Δ^9-tetrahydrocannabinol in rats.* PHARMAC. BIOCHEM. BEHAV. **2**(2) 227–233, 1974. – Intraperitoneal injections of Δ^8-tetrahydrocannabinol (THC) and Δ^9-THC, two major psychoactive constituents of cannabis, produced dose-related protection against tonic extension induced by electroshock in rats. The cannabinoids provided protection against clonic convulsions induced by pentylenetetrazol (Metrazol) only at very high and sometimes lethal doses, and the protection was quantal rather than dose-related. The two isomers of the THC were equipotent in terms of behavioral toxicity and protection against tonic convulsions. However, the significance of the drugs' anticonvulsant activity must be qualified by the observation that protection was provided by either drug only at doses producing marked toxic behavioral reactions.

Cannabis Δ^8-THC Δ^9-THC Anticonvulsant Seizure

A NUMBER of studies have indicated that naturally-occurring components of cannabis and synthetic cannabinoid drugs are capable of exerting antiepileptic effects (e.g. [1, 12, 17]). We have reported that low doses of Δ^9-tetrahydrocannabinol (Δ^9-THC), the presumed major psychoactive ingredient of cannabis [13], effectively antagonize seizures produced by localized subcortical electrical stimulation in freely-moving cats [21] and rats [6]. We have recently confirmed this effect in infrahuman primates (unpublished observations). The present paper extends our previous observations of the antiepileptic activity of Δ^9-THC and provides evidence concerning the antiepileptic activity of Δ^8-THC, a psychoactive isomer of THC [8]. We examined the neurotoxicity of the two cannabinoids and compared their effects on seizures induced by maximal electroconvulsive shock (MES) and by Metrazol (Met).

METHOD

Animals

Over 200 male hooded rats of the Royal Victoria Hospital strain weighing between 300–480 g were used. After arriving in the colony the rats were allowed 7 days to adapt to the colony environment before being tested. They were housed in groups of 4 in stainless steel cages with unrestricted access to food and water. On the day of the experiment each rat was transported to the laboratory and tested individually.

Cannabinoids

Stock solutions of each cannabinoid were supplied by the Department of Health and Welfare of Canada: Δ^8-THC was received in a solution of 99% ethanol at a concentration of 100 mg/ml and was reported to be 99% pure, while Δ^9-THC was received in a solution of absolute ethanol at a concentration of 200 mg/ml and was reported to be 95% pure. The purity of the drugs as reported by Health and Welfare was not verified in our laboratory. The stock solutions of the cannabinoids were further dissolved in propylene glycol and adjusted to yield a constant volume of 1 ml/kg for all but the highest doses of Δ^8-THC. Doses of Δ^8-THC 100 mg/kg or above were given dissolved in the stock solution with the volume proportional to the absolute amount of drug administered. The injections were given via the intraperitoneal route, and each rat received only one injection. Although poor absorption of cannabinoids has been reported following intraperitoneal administration (e.g., [9]), we used the i.p. route in the present experiment both for convenience and because we have previously

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observed dose-related effects of intraperitoneally-administered Δ^9-THC on seizure activity [6,21].

Maximal Electroshock

Electroconvulsive shock was generated from a power source consisting of two AC transformers wired in series and producing a peak output of 1100 V. A current of 50 mA and 0.7 sec in duration was delivered to the pinnae via miniature alligator clips wrapped in cotton and soaked in 0.9% saline. The usual seizure produced by electroshock in a nondrugged rat consists of loss of balance, a period of maximal tonic flexion and extension of the limbs, and then a period of generalized clonic jerking followed by immobility and apparent unconsciousness. The reliability of the seizure pattern was verified by subjecting each rat to at least two nondrug sessions before testing under the drug's influence.

An approximate ED50 for each cannabinoid was determined in preliminary experiments by injecting varying doses of the drugs (40–80 mg/kg) into groups of 4 rats each and testing the rats for protection from tonic extension induced by electroshock delivered 1 hr postinjection. The time of peak drug effect was then determined by injecting the approximate ED50 of each cannabinoid into 2 groups of 4 rats each and subjecting the groups to a staggered schedule of maximal electroshocks: The first group was shocked at 0.5 and 2 hr postinjection and the second group at 1 and 3 hr postinjection. The interval at which the greatest percentage of protection occurred was designated the time of peak effect for that cannabinoid, and all subsequent testing was performed at the peak time.

The ED50 for protection against electroshock-induced tonic extension was determined for Δ^8-THC and Δ^9-THC. Groups of 4 rats each received varying doses (15–125 mg/kg) of either cannabinoid and were tested for protection at the time of peak drug effect. In order to determine whether the potency of the 2 drugs differed significantly, a potency ratio was calculated (ED50 Δ^8-THC/ED50 Δ^9-THC as described by Litchfield and Wilcoxin [11]).

Metrazol-induced Seizure

Groups of 4 rats each received varying doses (15–200 mg/kg) of either cannabinoid. At the time of peak cannabinoid effect, the rats were given a subcutaneous injection of Metrazol into the dorsal surface of the neck at a dose of 70 mg/kg, the CD97 (the dose producing clonic convulsions in 97% of the rats [20]); and the incidence of clonic convulsions was recorded for 50 min after the Metrazol injection. The usual Metrazol-induced seizure in a nondrugged rat consists of a series of isolated myoclonic jerks which progressively increase in frequency and finally emerge into a fullblown generalized clonic convulsion.

Toxicity

A TD50 for each cannabinoid was determined by administering varying doses (1–9 mg/kg) of either drug to groups

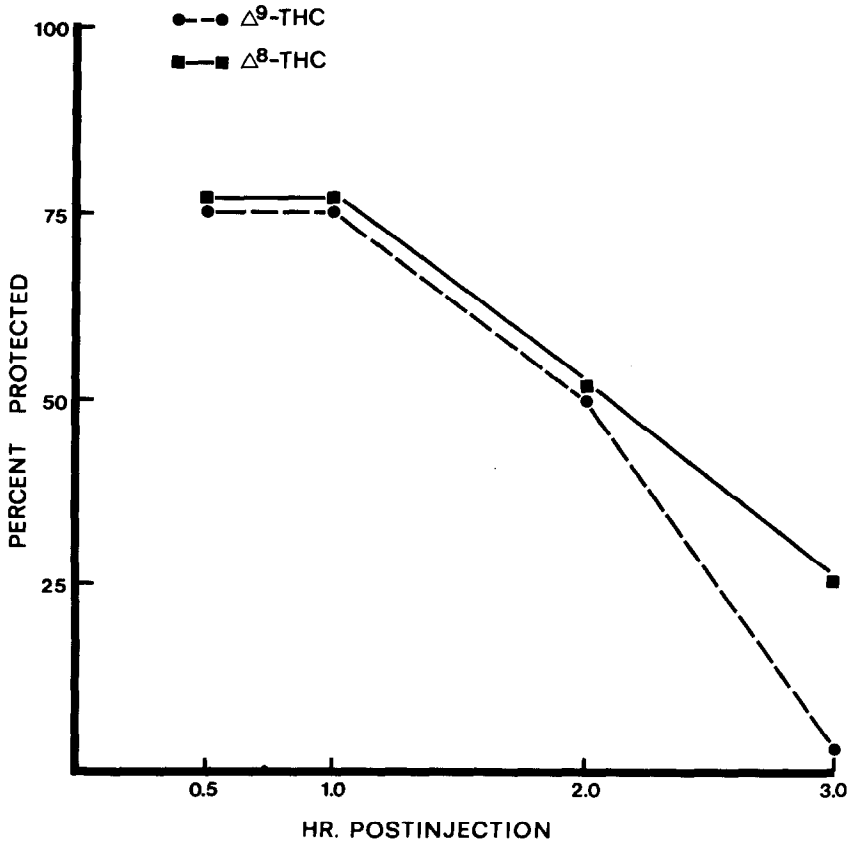

FIG. 1. Time to peak effect of Δ^8-THC and Δ^9-THC. Four rats per point.

of 4 rats each and then testing at the time of peak effect. The toxicity of a given dose was assessed by observing drug effects on the following measures [20]: gait, stance, muscle tone, positional sense (speed of retracting a hindlimb dangled over an edge), righting reflex, and equilibrium on a 3.0 × 100 cm suspended beam. Two observers examined each rat; one observer was unaware of the drug and dose that had been given to the rat. When both observers agreed that a given rat was responding abnormally on any of the above measures, that rat was considered to be displaying a toxic reaction to the drug. There was 100% agreement between the observers on the assessment of each rat's toxic response to the cannabinoids. A ratio of the potency of the 2 cannabinoids in the toxicity test was calculated [11].

RESULTS

The time of peak drug effect for both cannabinoids occurred at the 30 min and 60 min postinjection intervals, and the time-response relations were virtually identical for the 2 drugs (Fig. 1). Forty-five minutes was selected as the peak interval for all subsequent testing with either cannabinoid.

Maximal Electroshock

Δ^8-THC and Δ^9-THC each produced dose-related protection from the tonic extension induced by maximal electroshock, although the loss of balance, clonic jerking of the limbs, and postictal immobility produced by electroshock were not blocked by doses effective against tonic extension. The ED50s of Δ^8-THC and Δ^9-THC were 72.0 and 58.0 mg/kg respectively (Table 1). Zero and 100% protection occurred with each cannabinoid at respective doses of 15.0 and 125.0 mg/kg as shown in Fig. 2. Intermediate doses of Δ^8-THC (40.0, 60.0, 80.0, and 100.0 mg/kg) produced degrees of protection similar to those produced by the corresponding doses of Δ^9-THC. The potency ratio for activity in this test is 1.2, and the drugs do not differ significantly in their potency of protection against electroshock-induced extension ($p>0.05$).

Metrazol-induced Seizure

Both cannabinoids afforded some protection against the clonic convulsion produced by subcutaneous Metrazol. However, a graded dose-response relation was not apparent (Fig. 3), and only the highest doses produced reliable (i.e. >25%) protection against the seizures. Zero and 100% protection were obtained with each cannabinoid at respective doses of 15.0 and 200.0 mg/kg, as shown in Fig. 3. However, 4 of 4 rats receiving 200 mg/kg of Δ^8-THC died within 24 hr postinjection. The quantal nature of the dose-response relations is indicated by the fact that intermediate doses of each cannabinoid produced similar minimal degrees of protection. Because of the quantal nature of the dose-response relations and the fact that maximum protection was obtained only with extremely high and sometimes lethal doses of the cannabinoids, the data from the Metrazol study were not subjected to statistical analysis except in order to correct the zero and 100% values graphed in Fig. 3.

Toxicity

Toxic reactions to both cannabinoids were obtained at doses considerably below those protecting against maximal electroshock-induced or Metrazol-induced seizures. Neither drug produced toxic effects at a dose of 1.0 mg/kg, whereas 100% toxicity was obtained at 9.0 mg/kg (Fig. 4). The intermediate doses of Δ^8-THC (3.0, 5.0, and 7.0 mg/kg) produced percentages of toxic reactions similar to the corresponding doses of Δ^9-THC. The TD50s of Δ^8-THC and Δ^9-THC were 4.3 and 3.4 mg/kg respectively, as shown in Table 1. The potency ratio is 1.3 and there is no significant difference in the potency of the drugs in this test ($p>0.05$). The common effects produced by a toxic dose of either drug were flaccid muscle tone; mild to severe ataxia; catalepsy; drowsiness; vocalization, urination, and defecation in response to handling; hyperreactivity to noise or movement; delayed retraction of hindlimb in the position sense test; inability to right quickly; and lack of maintained balance on the suspended beam. Since the doses of each cannabinoid examined in the electroshock and Metrazol studies were considerably greater than their respective TD50s, severe toxic reactions were also observed in the animals in these studies. Although no attempt was made to characterize the toxic syndrome exactly, the obvious symptoms were extreme hypoactivity, catalepsy, and hypotonia.

TABLE 1

SUMMARY OF THE ANTICONVULSANT ACTIVITY OF Δ^8-THC AND Δ^9-THC

	MES ED50 in mg/kg*	TOXICITY TD50 in mg/kg*
Δ^8-THC	72.0 (48.0–108.0)	4.3 (2.7–6.8)
Δ^9-THC	58.0 (36.2–92.8)	3.4 (2.3–5.1)
Potency Ratio	1.2† (0.6–2.5)	1.3† (0.7–2.3)

*Parentheses contain the 95% confidence limits [11].
†No significant difference between Δ^8-THC and Δ^9-THC ($p>0.05$).

Control Studies with Ethanol Vehicle

Because the solutions containing the higher doses of the cannabinoids (especially Δ^8-THC) also contained large volumes of ethanol, it is possible that some of the anticonvulsant effects obtained at these high drug doses were a function of the depressant effects of the ethanol vehicle. To test this hypothesis 6 additional groups of rats were treated with volumes of 99% ethanol equivalent to the volumes of ethanol contained in the more concentrated cannabinoid solutions. Three groups of 4 rats each were given subcutaneous Metrazol (70 mg/kg) 45 min after an i.p. injection of 2.0, 1.5, or 1.0 ml/kg of 99% ethanol; note that 2 ml/kg is the largest volume of ethanol used to dissolve either cannabinoid in the Metrazol-induced seizure study. Three other groups of rats were exposed to maximal electroshock

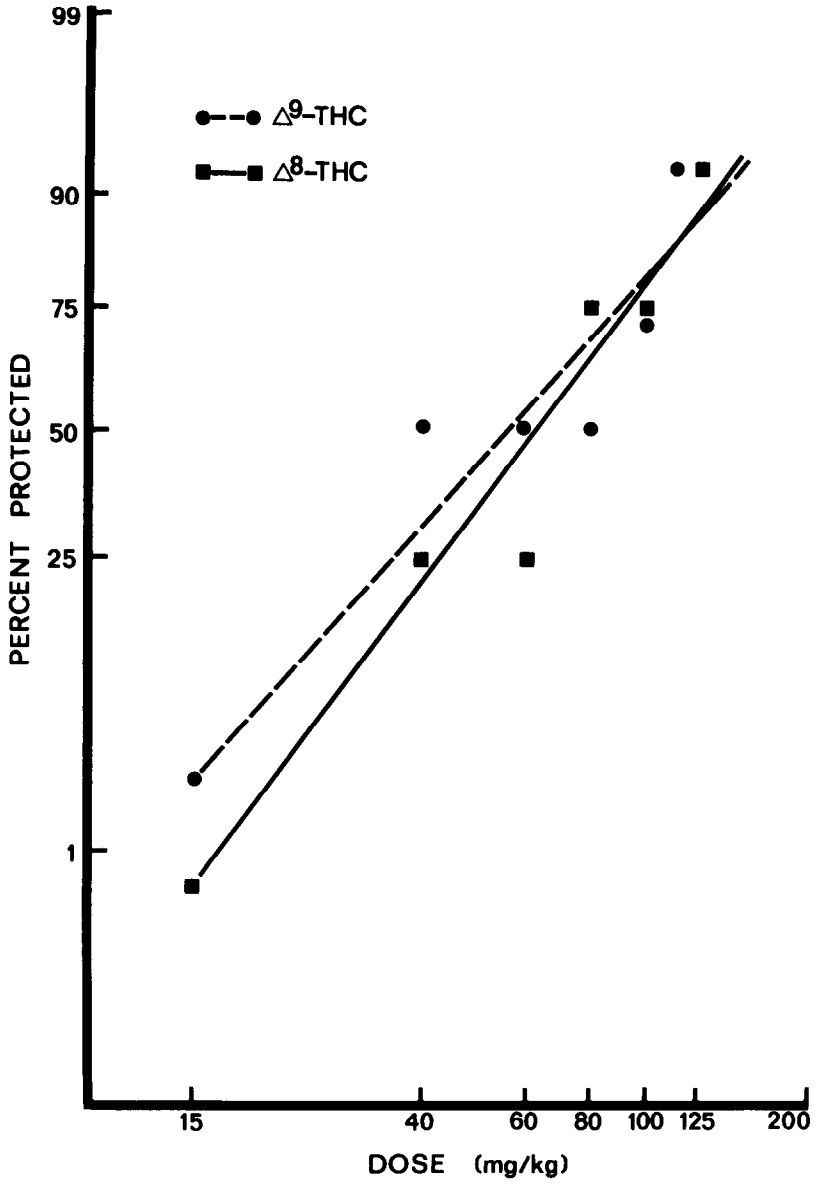

FIG. 2. Dose-response relations of the protection afforded by Δ^8-THC and Δ^9-THC against electroshock-induced tonic extension. Lines were fitted and zero and 100% values corrected according to the method of Litchfield and Wilcoxin [11]. Four rats per point.

45 min after an i.p. injection of 1.25, 1.0, or 0.85 ml/kg of 99% ethanol; 1.25 ml/kg is the largest volume of ethanol used to dissolve either cannabinoid in the electroshock-induced seizure study. Although all doses of ethanol produced marked toxic behavioral reactions including ataxia and hypoactivity, no dose produced any effect on either the Metrazol-induced or the electroshock-induced seizures: no protection was provided by the volume of ethanol present in the most concentrated solutions of cannabinoids tested. Since less than 0.05 ml of absolute ethanol was contained in the highest dose of the cannabinoids (9 mg/kg) examined in the toxicity test, it is unlikely that ethanol contributed to the toxic behavioral effects observed following administration of the lower doses of the cannabinoids. In support of this conclusion, we have confirmed in an unpublished study that an i.p. injection of this volume of absolute ethanol has no effect on the performance of rats in the toxicity test battery. The predominantly propylene glycol vehicle used with the lower concentrations of drug is not likely to have contributed to either the anticonvulsant or the toxic behavioral effects obtained, since the greatest volume of propylene glycol was contained in the least concentrated, nontoxic, and ineffective solutions of drug.

DISCUSSION

The present experiment confirms previous reports that cannabinoid drugs possess some degree of anticonvulsant

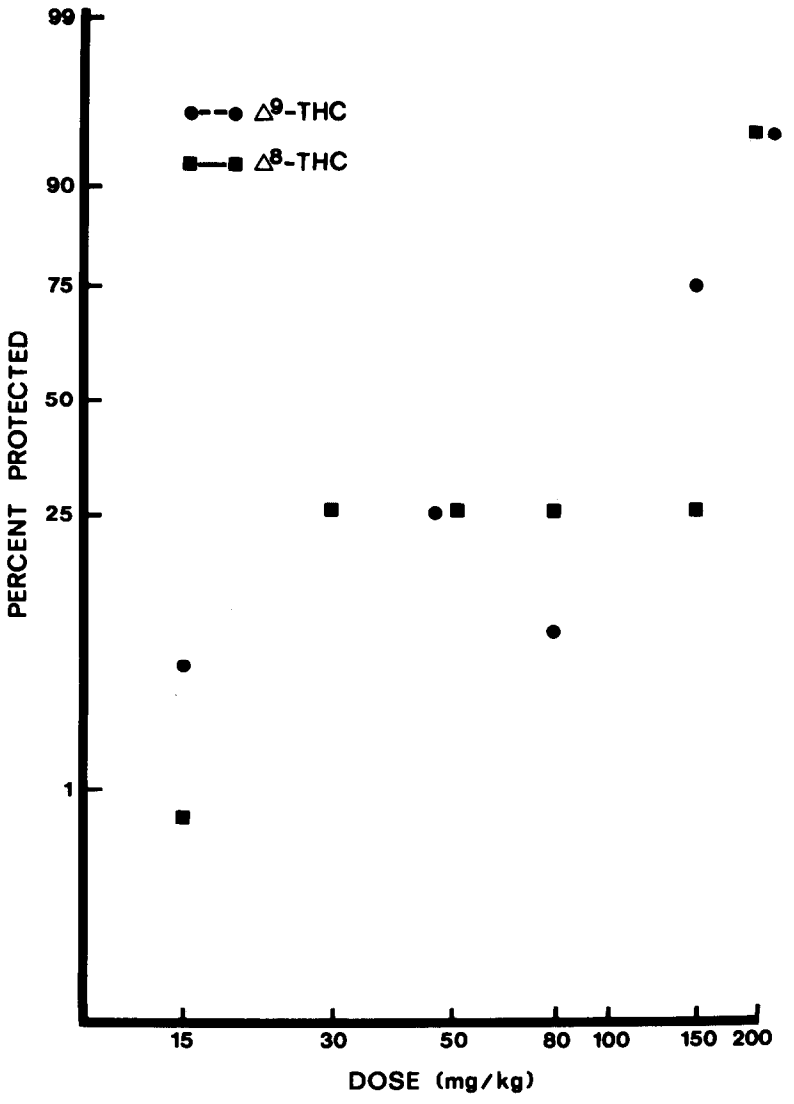

FIG. 3. Dose-response relations of the protection afforded by Δ^8-THC and Δ^9-THC against metrazol-induced clonic convulsion. Zero and 100% values were corrected [11] for the sake of comparison to the other figures. Four rats per point.

activity (e.g., [1,12]). The ED50 of Δ^9-THC against electroshock-induced tonic extension in rats reported here, 58.0 mg/kg, is in close agreement with the ED50 against electroshock-induced seizures following i.p. administration in mice, 54.5 mg/kg [17]. In contrast to the report of Sofia *et al.* that Δ^9-THC enhances Metrazol-induced clonic convulsions, we found that both cannabinoids have little effect on this type of seizure until extremely high (and, in the case of Δ^8-THC, lethal) doses are reached, at which point anticonvulsant effects are obtained. The two studies are in agreement that Δ^9-THC does not produce dose-related protection from Metrazol-induced clonic convulsions, and taken together lead to the conclusion that neither isomer of THC administered i.p. produces significant anticonvulsant effects against this type of seizure. There was no significant difference in the potency of the two isomers of THC on the measures reported here, although Δ^9-THC consistently produces comparable effects at somewhat lower doses than Δ^8-THC. Δ^8-THC is generally found to be less potent than Δ^9-THC in behavioral situations (e.g., [8]), but the present results suggest that this difference in potency does not extend to all of the drugs' behavioral effects.

It is not clear from the present results what might be the mechanisms underlying the protection against tonic seizures afforded by Δ^8-THC and Δ^9-THC. Control studies indicate that depressant effects of the ethanol contained in the vehicle cannot account for the anticonvulsant effects of the cannabinoids, since injection of the vehicle alone had no effect on either type of seizure studied here. These results also rule out the possibility that vehicle-induced tissue destruction (hemolysis or ulceration) or painful irritation at the site of injection account for the observed anticonvulsant effects, although such side effects could con-

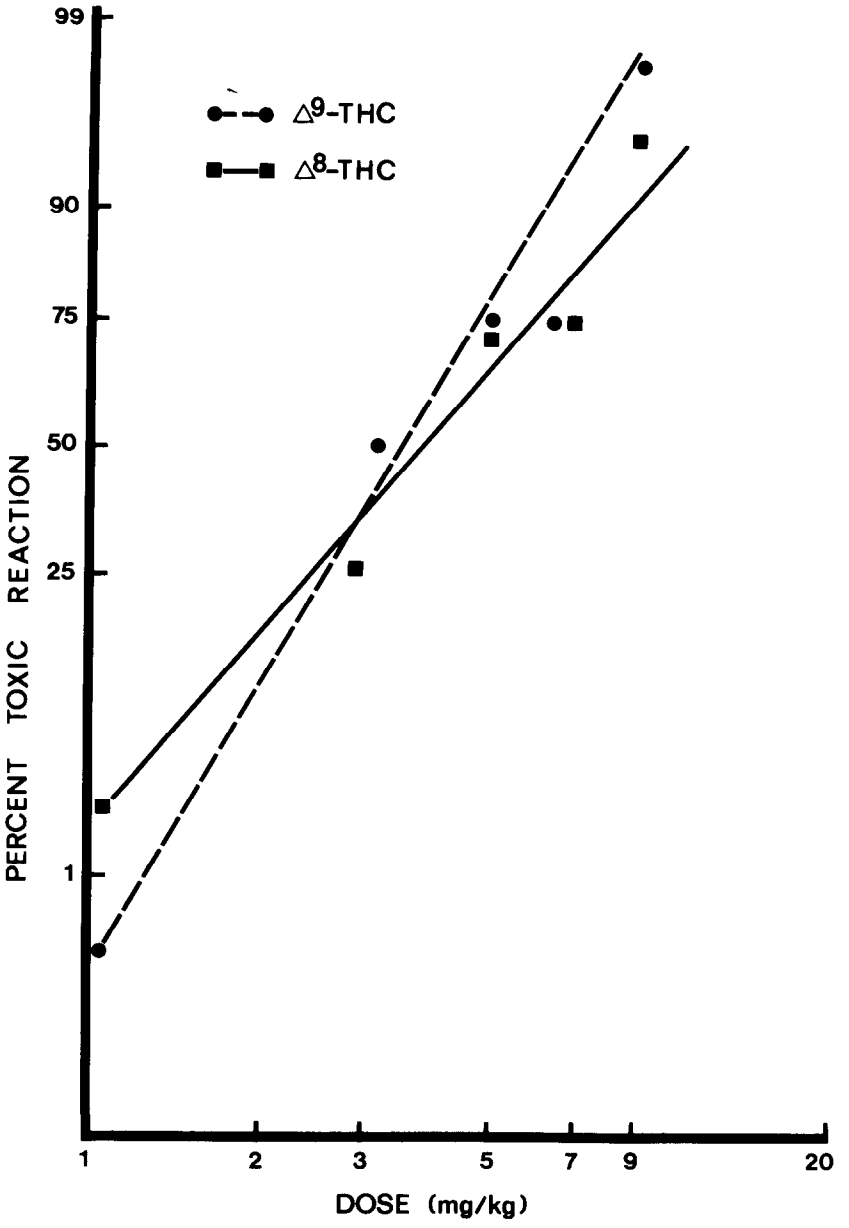

FIG. 4. Dose-response relations of Δ^8-THC and Δ^9-THC in producing toxic behavioral reactions. Lines were fitted and zero and 100% values corrected [11]. Four rats per point.

ceivably contribute to the toxic syndrome observed with high doses of the cannabinoids. It is possible that aversive effects produced by the cannabinoids themselves interfered with tonic seizures: Aversive footshock can block tonic extension induced by electroshock [14]; and cannabinoid drugs can produce aversive or unpleasant effects, as demonstrated by the fact that rats learn to avoid novel tastes that have been paired with injections of hashish [4], Δ^9-THC (7), Δ^8-THC (Bolotow, Corcoran, Amit, and McCaughran, in preparation), and other cannabinoids (Bolotow *et al.*). However, strong anticonvulsant effects were obtained in the present study only with doses of the THC isomers considerably above the minimal doses producing strong conditioned taste aversions in rats, suggesting that any aversive properties of the drugs are not sufficient to account for their effects on seizures. The hypothermia produced by high doses of THC [16] might be another factor contributing to the cannabinoids' effects on electroshock-induced seizures. Since hypothermia has been reported to increase susceptibility to electroshock [19], however, this property of the drugs would actually be expected to interfere with their anticonvulsant activity, and might account for the fact that relatively high doses of each isomer were required to block electroshock-induced tonic extension. An effect on cerebral catecholamines is another possible mechanism by which cannabinoids might exert

anticonvulsant effects: There is some evidence indicating that Δ^9-THC can increase turnover of cerebral norepinephrine [3, 10, 15], and both norepinephrine and dopamine have been implicated in the suppression of various types of seizures (e.g., [2,5]).

It is important to recognize that the protection against electroshock-induced tonic extension reported here was obtained only with relatively high doses of either drug. In contrast, toxic effects were seen at much lower doses, and the protective effects were thus accompanied by symptoms of gross behavioral toxicity. Since potential antiepileptic drugs that prove to be clinically useful generally have anticonvulsant effects in animals at doses that are below those producing toxic manifestations [18], the present experiment may indicate that Δ^8-THC and Δ^9-THC will be of little if any value as clinical antiepileptic drugs. This conclusion can best be verified, of course, in clinical trials. The present results are in some contrast to our previous reports that seizures produced by localized electrical stimulation of the brain can be suppressed by relatively low and in some cases nontoxic doses of Δ^9-THC [6,21]. Although there are a number of possible explanations for this discrepancy, the present experiment at a minimum suggests the caution that conclusions concerning the anticonvulsant properties of a drug may be valid only with reference to the specific measures used to characterize the drug's effects.

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