ACUTE EFFECTS OF Δ^8- AND Δ^9-TETRAHYDROCANNABINOL ON EXPERIMENTALLY-INDUCED SEIZURES

by

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ABSTRACT

Evaluations of dose-response effects of Δ^8- and Δ^9-tetrahydrocannabinol on three experimentally-induced seizure models, i.e., audiogenic seizure test, maximal electroshock seizure (MES) test and pentylenetetrazol-induced seizure test were carried out in the audiogenic rat. Results of this study indicate that both tetrahydrocannabinols possess a dose-related anticonvulsant effect against sound-induced seizure, maximal electroshock seizure and pentylenetetrazol-induced maximal seizure. Moreover, anticonvulsant potencies of Δ^8-tetrahydrocannabinol were not significantly different from that of Δ^9-tetrahydrocannabinol. These compounds were without effects on minimal chemoshock seizure and lethality induced by pentylenetetrazol. Furthermore, Δ^8-tetrahydrocannabinol is three times more neurotoxic (as measured by muscular incoordination) than Δ^9-tetrahydrocannabinol. The overall results of this investigation suggest that the anticonvulsant spectrum of activity of tetrahydrocannabinols resemble that reported for diphenylhydantoin.

CHAPTER 1

INTRODUCTION

Marihuana (Cannabis sativa) was first introduced into Western medicine by W. B. O'Shaughnessy (1840) who utilized hemp extract to treat many conditions including convulsions. Since that time, a few short communications have reported the anticonvulsive effect of marihuana and its derivatives. Loewe and Goodman (1947) were the first investigators to study the anticonvulsant properties of marihuana extract and Δ^3-tetrahydrocannabinol (Δ^3-THC), a synthetic analog of the active chemical derivatives of cannabis, in experimental animals. They reported that marihuana extract and Δ^3-THC abolished the hindleg tonic extensor component of maximal electroshock convulsion in rats but were ineffective against seizures induced by pentylenetetrazol. Sofia, Solomon and Barry (1971) observed that Δ^9-tetrahydrocannabinol (Δ^9-THC), an active chemical constituent of cannabis, protected mice against minimal and maximal electroshock seizure, but enhanced pentylenetetrazol-induced convulsions and lethality. Both tetrahydrocannabinols, Δ^3-THC and Δ^9-THC were reported by the respective groups of investigators to enhance the lethality of pentylenetetrazol. More recently, Fujimoto (1972) reaffirmed the anticonvulsant effect of Δ^9-THC against maximal electroshock in mice and Killam and Killam (1972) demonstrated that Δ^9-THC protects baboons (Papio papio) against photically induced myoclonic seizures.

As expected, a survey of the medical literature revealed almost a complete lack of clinical evaluations of the anticonvulsant property of marihuana and related drugs. Shortly after Loewe and Goodman reported their observations, Davis and Ramsey (1949) described the clinical use of Δ^3-THC in the management of grand mal epilepsy cases which were refractory to diphenylhydantoin and phenobarbital. Five such patients were treated with Δ^3-THC and it was reported that the seizures were brought under control. However, on the basis of one case history of an epileptic patient who used marihuana Keeler and Reifler (1967) suggested that marihuana may be hazardous to seizure-prone individuals.

There is a glaring paucity of published data on the anticonvulsant properties of marihuana and related drugs. Of the four animal studies cited above, three were published as abstracts. The present investigation was undertaken to compare the anticonvulsant properties of Δ^8-tetrahydrocannabinol (Δ^8-THC) and Δ^9-THC, two naturally occurring chemical constituents of marihuana, on three experimental models for epilepsy, i.e., audiogenic, maximal electroshock, and pentylenetetrazol-induced seizures. Toxicity, as indicated by neuromuscular incoordination, was also determined in order to calculate the protective index (i.e., TD50/ED50) of the two cannabinols. Because there is no published data on the enhancing effect of marihuana and related drugs on pentylenetetrazol lethality, Δ^9-THC was also quantified for this potential effect.

CHAPTER 2

GENERAL PROCEDURES

Experimental Animals

Adult female animals from the University of Arizona colony of audiogenic seizure-susceptible rats were used in these studies. Animals were allowed free access to food and water, except during the brief period of testing. Rats were housed, ten per cage, in a room maintained at a constant temperature of $25 \pm 2^{\circ}C$ and under controlled lighting, i.e., an equal cycle of light (0600-1800 hours) and darkness (1800-0600 hours).

Drugs

Injectable tetrahydrocannabinols were prepared according to the method described by Phillips, Turk and Forney (1971). Briefly, stock solutions of tetrahydrocannabinol (100 mg of tetrahydrocannabinol per vial) in ethanol were stored at $-10^{\circ}C$ and protected from light. Immediately prior to use, vehicular ethanol was evaporated and the liquid residue of tetrahydrocannabinol was then mixed with polysorbate (Tween) 80. The mixture was shaken vigorously on a vortex shaker. Isotonic saline solution was then added dropwise and vigorously shaken until a stable oil in water emulsion resulted. The final vehicle consisted of 10 percent polysorbate 80 in saline.

Solutions of 1.5 percent pentylenetetrazol were made up in isotonic saline solution just prior to being used.

Route of Administration

The tetrahydrocannabinols (Δ^8-THC and Δ^9-THC), pentylenetetrazol and vehicle of 10 percent polysorbate 80-saline were injected via the lateral tail vein in all animals.

Methods and Techniques

Audiogenic Seizure Test

Apparatus. A cylindrical sound chamber, made of galvanized metal having a diameter of 16 inches and a height of 20 inches, was utilized for audiogenic testing. Two electric doorbells mounted at the top of the chamber provided a sound level of approximately 115 decibles relative to 2×10^{-4} dyne/cm^2.

Methods. Within 15 seconds after an individual rat was placed in the chamber, sound was initiated and continued until the onset of clonus or tonus. If the animals failed to respond to the stimulus, i.e., absence of running and convulsion, sound was discontinued at the end of 120 seconds.

Audiogenic Response Score (ARS). Usually about 30 seconds after the initiation of sound stimulus, untreated audiogenic rats undergo two wilding running episodes (preconvulsive phase) followed by a clonic and/or tonic convulsion (convulsive phase). These running episodes are separated by a refractory period. Occasionally, the preconvulsive phase consists of one or rarely three running episodes.

The convulsive phase is characterized by a generalized clonus and/or tonus. The intensity of audiogenic seizure is recorded as a ranked audiogenic response score or ARS so that a comparison between test and control animals can be made. The scoring system described by Jobe, Picchioni and Chin (1973, pp. 1-10) is as follows:

ARS	Response to sound stimulation
0 =	no response, i.e., absence of convulsion and running.
1 =	running only, but no convulsion.
2 =	minimal seizure characterized by generalized clonus involving forelimbs, hindlimbs, pinnae and/or vibrissae; convulsion preceded by two running episodes separated by a refractory period.
3 =	same as 2, except only one running phase and no refractory period.
4 =	½ maximal seizure characterized by tonic flexion of neck, trunk, shoulder joint with tonic extension of elbow joints and clonus of pelvic limbs; convulsion preceded by two running episodes separated by a refractory period.
5 =	same as 4, except only one running phase and no refractory period.
6 =	3/4 maximal seizure resembles 4 except hindlimbs are in partial tonic extension (i.e., tonic extension of thighs and legs with clonus of feet); convulsion preceded by a refractory period.
7 =	same as 6, except one running phase and no refractory period.
8 =	maximal seizure resembles 4 except hindlimbs are in complete tonic extension; convulsion preceded by two running episodes separated by a refractory period.
9 =	same as 8, except one running phase and no refractory period.

In occasional animals which displayed three running episodes in the preconvulsive phase, 1/2 point was deducted from the audiogenic response score.

Experimental Procedures. Four or five days prior to experimentation, all animals were screened at least twice on different days to assure that each rat maintained a consistent ARS. Only rats that maintained an ARS of 5 or above were used in these studies. Two days after the final screening animals were randomly divided into groups of ten subjects each. Graded doses of Δ^8-THC, Δ^9-THC, or 10 percent polysorbate 80-saline vehicle was injected intravenously into each group of animals. Audiogenic seizure test was performed 30 minutes following injection and the ARS was recorded for each animal.

Statistical Procedures. The data obtained from the audiogenic seizure test were subjected to two different forms of statistical analyses. The median protective dose (ED50) of each drug was determined graphically, the 95 percent fiducial limits were calculated, and a comparison of the relative potencies of Δ^8-THC and Δ^9-THC was made according to the method of Litchfield and Wilcoxon (1949). In addition the effects of the tetrahydrocannabinols on the ranked ARS were evaluated by analysis of variance and the Student "t" test (Snedecor and Cochran 1967).

Maximal Electroshock Seizure (MES) Test

MES. A supramaximal current of 150 mA with a duration of 0.2 seconds supplied from an electroshock seizure apparatus was delivered through a pair of corneal electrodes to the eyes of the rat (Swinyard, Brown and Goodman 1952; Woodbury and Davenport 1952). Drops of isotonic saline were applied to the eyes of the rat prior to insertion of corneal electrodes to assure adequate electrode contact (Swinyard et al. 1952).

Experimental Procedures. Since not all rats respond with maximal convulsion after supramaximal electroshock (Swinyard et al. 1952) animals were screened three days before the experiment and only those which exhibit maximal electroshock seizure were used in the present studies. Graded doses of Δ^8-THC, Δ^9-THC or 10 percent polysorbate 80-saline vehicle was administered intravenously to each group of ten animals. Thirty minutes after injection, animals were tested for their response to supramaximal electroshock. The protective end point was abolition of the hindleg tonic extensor component of the seizure.

<u>Statistical Procedures</u>. The median protective dose (ED50) of each drug was determined graphically, the 95 percent fiducial limits were calculated, and a comparison of the relative potencies of Δ^8-THC and Δ^9-THC was made according to the method of Litchfield and Wilcoxon (1949).

Pentylenetetrazol Infusion Test

<u>Pentylenetetrazol Infusion</u>. The intravenous infusion technique as described by Orloff, Williams and Pfeiffer (1949) as modified by McQuarrie and Fingl (1958) was used for the pentylenetetrazol infusion test. The optimal rate of infusion, as suggested by Fingl and McQuarrie (1960) was 0.3 ml/minute (equivalent to 4.5 mg/minute) in this study.

<u>Experimental Procedures</u>. Graded doses of Δ^8-THC, Δ^9-THC or 10 percent polysorbate 80-saline vehicle was administered intravenously to each group of animals weighing between 180-195 grams. Thirty minutes after intravenous injection, each animal was infused with 1.5 percent pentylenetetrazol solution. Three end points are typically observed in the following sequence during continuous infusion: minimal seizure,

maximal seizure and death. A minimal seizure is defined as the persistent clonus of hindlimbs and/or forelimbs for at least three seconds. The infusion was terminated at the onset of maximal seizure (tonic extension of the hindlimbs). If an animal failed to display a maximal seizure, infusion was continued until the death of that animal. The infusion times for the induction of minimal and maximal seizures were recorded. The doses (or the concentration of pentylenetetrazol) of infusion was calculated from the following formula:

$$\text{Dose} = \frac{\text{infusion time (minutes)} \times 4.5 \text{ mg/minute}}{\text{weight of animals (kilogram)}}$$

Statistical Procedures. The data obtained were evaluated by analysis of variance (Snedecor and Cochran 1967).

Neurotoxicity Test

Apparatus. The rotorod technique described by Dunham and Miya (1957) was used in this study. A wooden rod, 36 inches in length and 1-1/8 inch in diameter was held 24 inches above the table top by two metal ring stands on each end. Several circular pieces of cardboard were placed on the rod equidistance from each other. These compartments restricted the walking space of the animals and allowed more than one rat to be trained at a time. The speed of rotation was adjusted to 15 revolutions per minute by means of a rheostat.

Experimental Procedures. Animals were trained "to walk" on a rotating rod until they could manage to remain on the rod for three one-minute trials.

Graded doses of the tetrahydrocannabinols or 10 percent polysorbate 80-saline vehicle were randomly given to each group of ten trained animals. Thirty minutes after injection, the ability of the animals "to walk" on the rod for one minute was tested. Animals which fell off the rotorod were considered to be neurotoxic.

Statistical Procedures. The median toxic dose (TD50) of each drug was determined graphically, the 95 percent fiducial limits were calculated, and a comparison of the relative potencies of Δ^8-THC and Δ^9-THC was made according to the method of Litchfield and Wilcoxon (1949).

Effect of Δ^9-THC on Pentylenetetrazol-induced Lethality

Experimental Procedures. Graded doses of 1.5 percent pentylenetetrazol solution were injected intravenously into the animals. Animals that died usually did so well within one hour, however, observations were made for one hour following injection of the convulsant.

Animals were randomly divided into groups of ten and a dose of 1.25 mg/kg of Δ^9-THC was injected intravenously into each rat. Thirty minutes later graded doses of pentylenetetrazol were injected intravenously and the number of deaths were recorded.

Statistical Procedures. The median lethal dose (LD50) of the drug with or without Δ^9-THC was determined graphically, the 95 percent fiducial limits were calculated, and a comparison of the relative potencies of pentylenetetrazol was made according to the method of Litchfield and Wilcoxon (1949).

CHAPTER 3

RESULTS

Audiogenic Seizure Test

The anticonvulsant effect of Δ^8-THC and Δ^9-THC against sound-induced convulsions was demonstrable whether quantitative or quantal end points were employed, i.e., ranked ARS or ED50, respectively (Table 1). The tetrahydrocannabinols were effective in reducing the ARS in all doses tested and exhibited a dose-response relationship ($F = 3.50$, $df = 29$, $p < 0.05$ for Δ^8-THC and $F = 8.20$, $df = 29$, $p < 0.05$ for Δ^9-THC). On the basis of complete protection from convulsions the ED50 calculations indicate that the median protective dose of Δ^8-THC is 6.50 mg/kg and that of Δ^9-THC is 3.30 mg/kg. However, despite the two-fold difference in the ED50s of Δ^8-THC and Δ^9-THC, a statistical evaluation of the relative potencies of the two tetrahydrocannabinols indicate that the two ED50s are not significantly different from each other ($p > 0.05$).

Maximal Electroshock Seizure (MES) Test

The tetrahydrocannabinols, Δ^8-THC and Δ^9-THC, are effective against maximal electroshock seizure as indicated by their ability to abolish tonic hindleg extension. All doses of the two drugs protected rats against MES (Table 2). Although Δ^8-THC appears to be twice as

Table 1. Effect of Δ^8- and Δ^9-tetrahydrocannabinol (Δ^8- and Δ^9-THC) on Sound-induced Seizure in the Audiogenic Rat.

Treatment	Number of Animals	Dose of THC mg/kg	Mean ARS $\pm$ S.E. Before (control)	After	% complete seizure-protection	ED50 mg/kg (95% C.L.)
10% polysorbate 80 - saline	10	-	6.15 $\pm$ 0.81	6.15 $\pm$ 0.98	0%	-
Δ^8 - THC	10	1.25	7.10 $\pm$ 0.57	4.50 $\pm$ 0.97*	10%	6.50 (3.50-11.83)
	10	2.50	6.20 $\pm$ 0.51	3.40 $\pm$ 0.86*	10%	
	10	5.0	5.37 $\pm$ 0.37	1.55 $\pm$ 0.45*	40%	
Δ^8 - THC	10	1.25	7.50 $\pm$ 0.54	4.00 $\pm$ 0.86*	10%	3.30 (2.00-5.45)
	10	2.50	5.85 $\pm$ 0.56	1.70 $\pm$ 0.42*	40%	
	10	5.0	7.22 $\pm$ 0.83	0.70 $\pm$ 0.37*	70%	

* Significantly different from matching control (paired Student "t" test, $p < 0.005$).

Table 2. Effect of Δ^8- and Δ^9-tetrahydrocannabinol (Δ^8- and Δ^9-THC) on Maximal Electroshock Seizure (MES) in the Audiogenic Rat.

Treatment	Number of Animals	Dose mg/kg	% Animals Protected	ED50 mg/kg (95% C.L.)
Δ^8-THC	10	1.25	10%	2.60 (1.57-4.29)
	10	2.50	60%	
	10	5.0	80%	
Δ^9-THC	10	1.25	20%	5.60 (2.66-11.76)
	10	2.50	40%	
	10	5.0	40%	

potent as Δ^9-THC, statistical evaluation of their relative potencies indicates that the two ED50s are not significantly different from each other ($p > 0.05$).

Pentylenetetrazol Infusion Test

The tetrahydrocannabinols, Δ^8-THC and Δ^9-THC and the vehicle did not decrease the infusion time for onset of minimal chemoshock convulsions ($p > 0.05$), however they were effective in reducing the incidence of maximal chemoshock convulsions (Table 3). A statistical evaluation of the effect of the tetrahydrocannabinols on maximal convulsion was not made because the experimental design of the

Table 3. Effect of Δ^8- and Δ^9-tetrahydrocannabinol (Δ^8- and Δ^9-THC) on Pentylenetetrazol (PTZ) Infusion in the Audiogenic Rat.

Treatment	Number of Animals	Dose of THC mg/kg	Minimal Seizure*		Maximal Seizure
			Onset Time $\pm$ S.E. sec	Convulsive dose of PTZ $\pm$ S.E. mg/kg	% Animals Protected
None	10	-	82.7 ± 2.50	33.32 ± 1.02	0%
10% polysorbate 80-Saline	10	-	85.5 ± 5.29	34.64 ± 2.13	30%
Δ^8-THC	10	1.25	70.3 ± 6.51	27.98 ± 2.63	40%
	10	2.50	80.9 ± 5.47	33.05 ± 2.25	80%
	10	5.0	69.8 ± 4.90	27.60 ± 1.83	80%
Δ^9-THC	10	0.05	79.4 ± 5.69	32.64 ± 2.32	60%
	10	0.10	74.5 ± 4.55	30.67 ± 1.90	50%
	10	1.25	77.2 ± 3.91	31.06 ± 1.44	80%

* None of the test values are significantly different from the control value ($p > 0.05$).

pentylenetetrazol infusion test is based on measurement of time required for the onset of specific endpoints and some tetrahydrocannabinol-treated animals did not have maximal seizures. However, the fact that Δ^9-THC and Δ^8-THC produced comparable incidences of protection against maximal convulsion despite the fact that a lower dosage range of Δ^9-THC was used, strongly suggests that Δ^9-THC is more potent than its Δ^8 analog in abolishing maximal pentylenetetrazol-induced maximal convulsions.

Neurotoxicity Test and Calculation of Protective Indexes

The neurotoxicity study suggests that Δ^8-THC is over three times more potent than Δ^9-THC in the production of neuromuscular deficit, as indicated by three-fold difference in the respective median toxic doses (Table 4). Statistical analysis revealed that this difference is significant ($p < 0.05$).

Calculation of the protective indexes (P.I.) of the tetrahydrocannabinols reveal that the P.I. of Δ^8-THC for audiogenic seizure and maximal electroshock seizure are both less than one (Table 5). The P.I. of Δ^9-THC for audiogenic seizure and maximal electroshock seizure are greater than one, but less than two.

Effect of Δ^9-Tetrahydrocannabinol on Pentylenetetrazol Lethality

The lethality of pentylenetetrazol is not increased or decreased by pretreatment with 1.25 mg/kg of Δ^9-THC (Table 6). Statistical comparison revealed no significant difference between the two LD50s ($p > 0.05$).

Table 4. Neurotoxic Effect of Δ^8- and Δ^9-tetrahydrocannabinol (Δ^8- and Δ^9-THC) in the Audiogenic Rat.

Treatment	Number of Animals	Dose mg/kg	% Neurotoxic	TD50 mg/kg (95% C.L.)
Δ^8-THC	10	0.50	10%	1.85 (0.98-3.45)
	10	2.75	70%	
	10	5.0	80%	
Δ^9-THC	10	5.0	30%	6.15 (5.0-7.56)
	10	7.5	80%	
	10	10.0	80%	

Table 5. Anticonvulsant Effect and Protective Indexes (P.I.)* of Δ^8- and Δ^9-tetrahydrocannabinol (Δ^8- and Δ^9-THC) on Audiogenic Seizure (AS) and Maximal Electroshock Seizure (MES) in the Audiogenic Rat.

Drug	TD50 mg/kg (95% C.L.)	AS		MES	
		ED50 mg/kg (95% C.L.)	P.I. (95% C.L.)	ED50 mg/kg (95% C.L.)	P.I. (95% C.L.)
Δ^8-THC	1.83 (0.98-3.45)	6.50 (3.50-11.83)	0.28 (0.12-0.67)	2.65 (1.57-4.29)	0.71 (0.32-1.59)
Δ^9-THC	6.15 (5.0-7.56)	3.30 (2.0-5.45)	1.86 (1.08-3.16)	5.60 (2.66-11.76)	1.09 (0.51-2.35)

* Protective Index: TD50/ED50.

Table 6. Effect of Δ^9-tetrahydrocannabinol (Δ^9-THC) on Pentylenetetrazol (PTZ)-induced Lethality in the Audiogenic Rat.

Treatment	Number of Animals	Dose of PTZ mg/kg	% Lethality	LD50 mg/kg (95% C.L.)
PTZ	10	20.0	10%	28.5 (24.56-33.06)
	10	27.5	40%	
	10	35.0	80%	
PTZ + 1.25 mg/kg Δ^9-THC	10	20.0	10%	30.4 (25.87-35.72)
	10	27.5	30%	
	10	35.0	70%	

Behaviors Observed in Tetrahydrocannabinol-treated Rats

In a dose range of 1.25-10.0 mg/kg the THCs caused depression as evidenced by catalepsy, ataxia and decreased spontaneous locomotion. This behavioral depression lasted for several hours. With higher dosage levels (i.e., 5 mg/kg or greater), periodic states of motor excitation such as jerking and jumping usually occurred within 15 minutes after injection. A lower dose of 2.5 mg/kg occasionally produced these symptoms. No clonic or tonic convulsions were observed at any doses used in the study. All animals were hyperactive to sound and tactile stimuli. Vocalization upon tactile stimulation was a characteristic behavior that was induced by low doses of the tetrahydrocannabinols, i.e., 0.05-2.50 mg/kg. A strong grasping movement in all four limbs and marked rigidity in the tail were also observed.

CHAPTER 4

DISCUSSION

The intravenous route was used for the administration of the tetrahydrocannabinols in this investigation because these chemicals are poorly absorbed following other routes of administration. For example, Ho et al. (1971) reported that tritiated Δ^9-THC administered intraperitoneally in polysorbate (Tween) 80 and saline yielded only approximately 10 percent absorption.

The test time of 30 minutes was employed because a pilot study in our laboratory indicated that 30 minutes is the peak time for the anticonvulsant effect of Δ^9-THC following intravenous injection (Man and Consroe 1973). It is of interest to note that Ho et al. (1971) reported that radioactivity was immediately detectable in the central nervous system following intravenous administration of tritiated Δ^9-THC and Klausner and Dingell (1970) reported that peak activity in the central nervous system occurred 30 minutes following intravenous injection of carbon labeled Δ^9-THC.

Another factor which greatly influences the pharmacology of tetrahydrocannabinol is the vehicle in which the drug is administered. Various substances have been used to solublize or suspend tetrahydrocannabinols in liquid form; solvents such as ethanol and propylene glycol or suspending agents such as surfactants (e.g., polysorbate 80) and bovine serum albumin are commonly employed. Organic solvents, such as ethanol, are undesirable because they contribute their own depressant effect to the pharmacology of tetrahydrocannabinols (Kubena and Barry 1970, Forney 1971). Bovine serum albumin has the major disadvantage that it has a strong affinity for binding the tetrahydrocannabinols and thereby reduce their pharmacological activity. All factors considered, the surfactant polysorbate 80 may be the best agent for rendering tetrahydrocannabinols miscible with water. Polysorbate 80 was employed in this investigation to prepare emulsion of tetrahydrocannabinols for injection, because it is believed to have negligible pharmacological activity.

The two tetrahydrocannabinols, Δ^8-THC and Δ^9-THC were observed to be effective in blocking audiogenic convulsions, maximal electroshock convulsions, and maximal pentylenetetrazol convulsions in a dose related manner. The anticonvulsant potencies of these two tetrahydrocannabinols, as measured by the above convulsive tests, are similar. This presentation is the first report of the anticonvulsant effect of tetrahydrocannabinols against audiogenic seizures and is the first report of the use of Δ^8-THC against any seizure models. The results of the maximal electroshock study support previous reports that marihuana extract and Δ^3-THC protect rats (Loewe and Goodman 1947) and that Δ^9-THC protects mice (Sofia et al. 1971) against maximal electroshock convulsions.

The pentylenetetrazol infusion study indicate that Δ^8-THC and Δ^9-THC do not protect rats against minimal pentylenetetrazol convulsions, an observation which coincides with the findings of Loewe and Goodman (1947) that marihuana extract and Δ^3-THC are ineffective in blocking seizures induced by subcutaneous injection of pentylenetetrazol. In contrast, the report of Sofia et al. (1971) that Δ^9-THC enhances pentylenetetrazol seizures and lethality in mice is at variance with the present data; neither pentylenetetrazol infusion time for maximal pentylenetetrazol seizure nor the LD50 of pentylenetetrazol in rats was affected by tetrahydrocannabinols. It should be noted that different species of rodents were employed in these studies and the difference in results may be attributable to the difference in species. It is all the more perplexing that Karler (1973) recently reported that Δ^9-THC protects mice against seizure produced by subcutaneous injection of pentylenetetrazol.

In general the tetrahydrocannabinols appear to be qualitative similar to diphenylhydantoin in its spectrum of anticonvulsant activity. Diphenylhydantoin is known to exert a potent anticonvulsant action on maximal audiogenic seizure (Merritt and Putnam 1938, Griffiths 1942, Fink and Swinyard 1959, Swinyard et al. 1963), maximal electroshock seizure (Toman and Goodman 1946, Gray et al. 1958, Fink and Swinyard 1959, Mitchell and Keasling 1960, Swinyard 1963, Swinyard et al. 1963) and maximal pentylenetetrazol-induced seizure (Mitchell and Keasling 1960) in mice and rats. However, even in high doses (100 mg/kg or more) diphenylhydantoin is not only ineffective in blocking minimal pentylenetetrazol seizure but enhances the seizure severity of the convulsant agent (Goodman and Lih 1941; Goodman, Swinyard and Toman 1946;

Chen, Bohner and Ensor 1954; Mitchell and Keasling 1960). Similarly, Δ^8-THC and Δ^9-THC are effective against maximal audiogenic, maximal electroshock and maximal pentylenetetrazol convulsions but not against minimal pentylenetetrazol convulsions. However, tetrahydrocannabinol did not enhance minimal pentylenetetrazol convulsions in the doses employed in the present study. According to the limited literature regarding the anticonvulsant activity of tetrahydrocannabinol, the similarities between diphenylhydantoin and tetrahydrocannabinol are not entirely clear cut. For example, Sofia et al. (1971) indicate that Δ^9-THC enhances pentylenetetrazol convulsions and lethality in mice, whereas Karler (1973) report that Δ^9-THC protected mice against subcutaneous injections of pentylenetetrazol.

The overt behaviors observed in the tetrahydrocannabinol-treated rats are in general agreement with those reported by other authors. Such behaviors include production of an initial excitation followed by depression after administration of high doses of tetrahydrocannabinol (Grunfeld and Edery 1969, Moreton and Davis 1970), hyperactivity to auditory and tactile stimuli (Grunfeld and Edery 1969, Truitt 1971), vocalization (Carlini and Kramer 1965, Henriksson and Jarbe 1971), ataxia (Grunfeld and Edery 1969, Holtzman et al. 1969, Irwin 1969, Truitt 1971) and catalepsy (Grunfeld and Edery 1969, Schildkraut and Efron 1971, Truitt 1971). Moreover, the powerful grasping ability and increased rigidity in the tail observed in the audiogenic rats suggest that muscle tone is not reduced in tetrahydrocannabinol-treated animals. Interestingly enough, these characteristic effects of tail rigidity have been reported in reserpine-treated rats (Cole and Dearnaley 1959).

In the present investigation Δ^8-THC was found to have the same pharmacological effects as Δ^9-THC. It is as effective as Δ^9-THC in the suppression of audiogenic and maximal electroshock seizures. It appears to be less potent than Δ^9-THC in the suppression of maximal pentylenetetrazol convulsions. In addition, Δ^8-THC is three times more neurotoxic than Δ^9-THC and its protective indexes are lower than those of Δ^9-THC for audiogenic and maximal electroshock seizures. It is of interest to note that Δ^8-THC and Δ^9-THC also have analgesic activity and that Δ^9-THC is reported to be more potent than Δ^8-THC on the basis of the hot plate test and the phenylquinone abdominal-stretching test in mice (Harris 1971; Dewey, Harris and Kennedy 1972). The tetrahydrocannabinols also depress responses to a schedule-controlled behavior of rats and pigeons, with Δ^9-THC being more effective in this regard (Frankenheim, McMillan and Harris 1971; Harris 1971). Thus, although Δ^8-THC and Δ^9-THC appear to be identical in their pharmacological effects it is inappropriate to generalize that the pharmacological potency of one tetrahydrocannabinol is greater or smaller than another tetrahydrocannabinol. Indeed, the pharmacological effects of both tetrahydrocannabinol compounds must be determined on each measured parameter before true potency comparisons can be made.

CHAPTER 5

SUMMARY AND CONCLUSIONS

1. The tetrahydrocannabinols are active against sound-induced and maximal electroshock seizure. No significant differences exist between the potencies of these two compounds in these tests.

2. Tetrahydrocannabinols are ineffective in protecting against pentylenetetrazol-induced minimal seizures. However, maximal pentylenetetrazol seizures are blocked by both Δ^8-THC and Δ^9-THC.

3. Delta-9-THC is three times less toxic than Δ^8-THC when measured by a test for muscular incoordination (rotorod test). Thus the protective indexes are greater for Δ^9-THC than for Δ^8-THC.

4. Pretreatment with Δ^9-THC, 1.25 mg/kg, has no effect on pentylenetetrazol-induced lethality in the audiogenic rat.

5. Comparison of the present data with previously published data on diphenylhydantoin indicates that Δ^8-THC and Δ^9-THC have a spectrum of anticonvulsant activity which is similar to that of the antiepileptic drug.

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