Life Sciences Vol. 15, pp. 931-947
Printed in the U.S.A.

Pergamon Press

ANTICONVULSANT PROPERTIES OF Δ^9-TETRAHYDROCANNABINOL AND OTHER CANNABINOIDS

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(Received in final form 22 July 1974)

Summary

Anticonvulsant doses of Δ^9-tetrahydrocannabinol (Δ^9-THC) markedly lower body temperature in mice at an ambient temperature of 22°C, but there is little such effect at 30°C. The anticonvulsant properties of Δ^9-THC are as follows: The drug abolishes hind-limb extension in a maximal electroshock (MES) test, elevates both the MES (extensor) and 6-Hz-electroshock thresholds, exerts no effect on the 60-Hz-electroshock threshold, and enhances minimal seizures caused by pentylenetetrazol. All anticonvulsant properties studied, with the exception of the 60-Hz-electroshock threshold, were unaffected by the hypothermia resulting at 22°C. Additional experiments with Δ^9-THC indicated that chronic treatment results in the development of tolerance, as determined by the MES test with rats. The four principal naturally occurring cannabinoids, Δ^9-THC, Δ^8-THC, cannabinol and cannabidiol, display anticonvulsant activity, as does the major, primary metabolite of Δ^9-THC, 11-hydroxy-Δ^9-THC. Of all agents investigated in mice, the synthetic cannabinoids, dimethylheptylpyran and its isomers, are the most potent anticonvulsants. The results of a study of the relative motor toxicity and anticonvulsant activity of the cannabinoids demonstrate that these properties are at least partially separable among the various agents.

Although the anticonvulsant activity of marihuana has been recognized for many years (1), more recent investigations have been directed towards the components of marihuana: Thus, the anticonvulsant properties of pure Δ^9-tetrahydrocannabinol (Δ^9-THC), one of the main

constituents of marihuana, have been described by a number of laboratories (2, 3, 4, 5, 6, 7, 8); and a variety of tests have been used to determine the anticonvulsant activity of other pure cannabinoids, both naturally occurring and synthetic (1, 9, 10, 11, 12). The notion, therefore, that cannabinoids may be useful antiepileptic agents is a logical one and, indeed, has been suggested by the clinical report of Davis and Ramsey (13); however, an obvious limitation to their clinical use is their central nervous system toxicity.

In order to assess the antiepileptic potential of the cannabinoids, a more detailed description of their properties is needed; accordingly, the present investigation had several objectives: The first was to measure the influence of an anticonvulsant dose of Δ^9-THC on body temperature; the second was to describe its anticonvulsant characteristics by a battery of conventional laboratory tests and to gauge the influence of body temperature on these results; the third was to study the development of tolerance to the effects of Δ^9-THC against maximal electroshock (MES); and the last objective was to survey several cannabinoids for their relative anticonvulsant activity and motor toxicity in order to learn if these two attributes are separable.

Methods

Experimental animals and drug preparations. The experiments were conducted on approximately 4-week-old male, Charles River mice (ICR) and Sprague-Dawley rats. Body weights for mice ranged from 15 to 26 gm and for rats, 75 to 95 gm. The method of Rosenkrantz et al. (14) was used to prepare emulsions of drugs for intraperitoneal injection. The final emulsion consisted of about 10% sesame seed oil, 1% Tween 80

and 89% saline solution. Δ^8-THC was mixed only with Tween 80 and was dispersed in isotonic saline solution; this emulsion contained about 8% Tween 80 and 92% saline and produced more consistent results than the corresponding preparation using sesame seed oil. Control animals received injections of the appropriate vehicle. The volumes of the injections were 0.1 ml/20 gm for mice and 0.1 ml/100 gm for rats.

Anticonvulsant tests. Anticonvulsant activity was measured against electrically caused convulsions in the following laboratory procedures: MES, MES threshold, 6-Hz-electroshock threshold (6-Hz-EST) and 60-Hz-electroshock threshold (60-Hz-EST). The two MES and the 60-Hz-EST tests were conducted with a constant current apparatus (15). In the 6-Hz-EST studies a Grass stimulator, modified to produce a constant voltage, was used. Stimuli were applied by means of conventional corneal electrodes. The end-points were abolition of hind-limb extension for the MES test, hind-limb extension (a maximal seizure) for the MES threshold test, and front-limb and jaw clonus (a minimal seizure) for both EST tests (15). Anticonvulsant activity was also measured against minimal seizures caused by subcutaneously administered pentylenetetrazol (PTZ) (15).

The time course of anticonvulsant effect was determined in the MES test by the use of the ratio of extensor-to-flexor time (E/F) (9). Anticonvulsant activity shortens the duration of MES-caused extension; hence, the greater the anticonvulsant activity, the lower is the E/F ratio. All experiments were carried out at the peak-effect time for each drug, as denoted by the test time with the lowest E/F ratio and the greatest number of animals protected against MES.

Some of the experiments were conducted at normal room temperature (22°C) and others were at 30°C. When they were performed at 30°C, all animals were allowed 1 to 2 hr for acclimatization to the higher-than-normal ambient temperature.

Motor-toxicity experiments. Toxicity was determined by a bar-walk test. If an animal was unable to walk a 49 x 1.3 cm steel rod in three attempts within a 2-min period, it was considered to have impaired motor function. Animals were subjected to the bar walk before the administration of either drug or vehicle and again just prior to the MES. We discovered that some normal mice were unable to walk the bar, so at the beginning of each experiment a preliminary test was included in order to screen out such animals. After treatment, the bar walk was repeated just prior to the MES, because the peak-effect times for motor toxicity and anticonvulsant activity coincided.

Experimental design and data analysis. Each dose-, current- and voltage-effect curve has three to six points; each point represents the results obtained from 12 to 20 animals. Median-effective values and their 95% confidence limits were determined by probit analysis (16); the calculations were made by a Univac computer 1108. Relative potency tests were carried out by the methods of Litchfield and Wilcoxon (17). Graphs were drawn by a Gerber plotter, with the exception of those from the tolerance studies.

Temperature determinations. Measurements of ambient and rectal temperatures were made with an electronic thermometer. Rectal temperatures were obtained by inserting a probe a constant distance, 2.5 cm.

Results

Effect of an anticonvulsant dose of Δ^9-THC on rectal temperature. Table I represents the results of Δ^9-THC administration (100 mg/kg) on the body temperature of mice at two different ambient temperatures, 22° and 30°C. A marked hypothermic response occurs at 22°C, but is almost abolished when the ambient temperature is 30°C.

TABLE I

Influence of Ambient Temperature on Hypothermia Caused by Δ^9-THC

Ambient Temperature (°C)	Mean Rectal Temperatures and Their Standard Deviations (°C)*	
	Control	Δ^9-THC-treated
22	37.4 ± 0.55	32.9 ± 1.0†
30	37.4 ± 0.32	36.8 ± 0.59

* Data were obtained from 60-Hz-EST study illustrated in Fig. 1; since rectal temperatures at 1 and 2 hr after Δ^9-THC were similar, measurements were made 1 hr after treatment with either vehicle or Δ^9-THC (100 mg/kg) to minimize a possible interference with the anticonvulsant test that was conducted 2 hr after treatment; each mean represents the results obtained from 15 mice.

† As indicated by analysis of variance and the Newman-Keuls multiple means test, the value is significantly different from the other three mean values ($P < 0.05$) (18).

Because of this data, subsequent experiments designed to characterize the anticonvulsant properties of Δ^9-THC were conducted at both temperatures in order to evaluate the contribution of body-temperature changes to the observed effects.

Anticonvulsant properties of Δ^9-THC. The effects of Δ^9-THC in various threshold experiments with mice at an ambient temperature of 30°C are summarized in Table II; typical data used to obtain all of the

median-effective values are illustrated in Fig. 1.

TABLE II

Effects of Δ^9-THC on Various Threshold Tests for Anticonvulsant Activity

	Median-effective Values and Their 95% Confidence Limits*	
Test†	Control	Δ^9-THC-treated
MES threshold	9.4 mA (8.4-11)	11 mA (9.9-13)‡
6-Hz-EST	39 V (36-42)	47 V (40-53)‡
60-Hz-EST	6.1 mA (5.7-6.5)	5.7 mA (5.5-5.9)
PTZ threshold	48 mg/kg (43-53)	39 mg/kg (34-45)‡

* Tests were conducted 2 hr after 100 mg/kg of Δ^9-THC or vehicle (ambient temp., 30° C).

† MES, maximal electroshock; EST, electroshock threshold; PTZ, pentylenetetrazol.

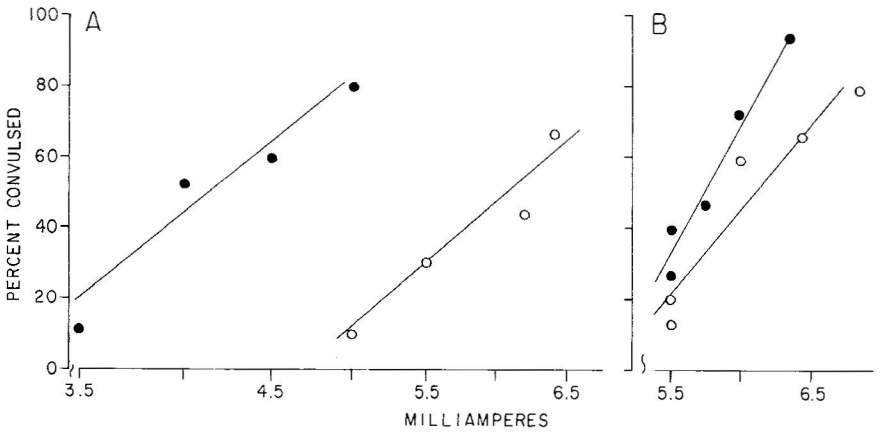
‡ Value significantly different from control, as indicated by a relative potency test ($P \leq 0.05$) (17).

Δ^9-THC, as depicted in Table II, raises both the MES threshold and the 6-Hz-EST; in contrast, it does not alter the 60-Hz-EST and lowers the PTZ threshold. Other investigators have also reported that Δ^9-THC enhances PTZ-caused minimal seizures in rats and mice (1, 3). Consroe and Man (11), however, found that in the rat Δ^9-THC does not affect minimal seizures produced by intravenously infuzed PTZ. The reason for the discrepancy in results is unclear, but it may be related to the use of different routes of administration of PTZ.

Additional threshold studies to determine the anticonvulsant effects of Δ^9-THC in hypothermic mice were performed at an ambient temperature of 22°C. Only the results of the 60-Hz-EST test were

affected by the Δ^9-THC-caused hypothermia. For example, in one

FIG. 1



Effects of Δ^9-THC on 60-Hz-EST. Percent convulsed is plotted against milliamperes on a logarithmic scale; curves were drawn by a plotter. Each point represents results from 15 mice. Control data are illustrated by unfilled circles; treated, by filled circles. In A the ambient temp. was 22°C; in B the ambient temp. was 30°C. Mean rectal temp. measured in these experiments are listed in Table I.

60-Hz-EST study at an ambient temperature of 22°C, the median-effective currents and their 95% confidence limits were 6.1 mA (5.5-6.7) for control and 4.2 mA (3.7-4.7) for treated mice (Fig. 1A). As determined by a test for relative potency (17), these median-effective currents are significantly different ($P \leq 0.05$), whereas comparable values (Table II, Fig. 1B) obtained from mice at an ambient temperature of 30°C are not significantly different ($P > 0.05$).

The influence of Δ^9-THC-induced hypothermia on the drug's

anticonvulsant activity against MES was also evaluated. The results of these experiments are shown in Table III and indicate that anticonvulsant activity, as evaluated by the E/F ratio, does not appear to be influenced by hypothermia.

TABLE III

Influence of Δ^9-THC-caused Hypothermia on the E/F Ratio from an MES Test*

Ambient Temperature (°C)	N**	Rectal Temperature†	E/F Ratio†
22	28	33.0 ± 1.3‡	2.0 ± 2.5‡
30	42	36.4 ± 1.0‡	2.4 ± 2.2‡

* Mice given 100 mg/kg of Δ^9-THC.

** N, number of mice.

† Mean values and their standard deviations.

‡ Rectal temperatures are significantly different, as determined by a t-test ($P < 0.001$).

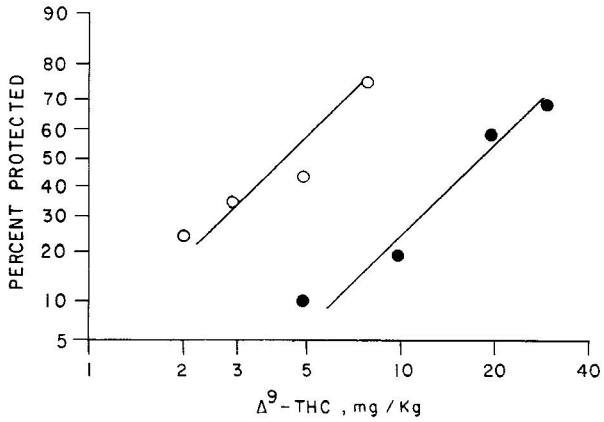
‡ E/F ratios are not significantly different, as determined by a t-test ($P > 0.40$).

Development of tolerance to the anticonvulsant effects of Δ^9-THC.

Rats were treated each day for seven days with Δ^9-THC (5 mg/kg/day); a control group was similarly treated with vehicle. On the eighth day both groups were given Δ^9-THC, and dose-effect curves were obtained in the MES test. The resulting data are illustrated in Fig. 2. The ED50 values and their 95% confidence limits were 4.1 mg/kg (2.7-5.9) for the acutely treated rats and 19 mg/kg (13-26) for the chronically treated group. The use of a relative potency test (17) indicated that the ED50 values are statistically different ($P \leq 0.05$). The results clearly demonstrate that

tolerance, as measured by the MES, can develop to the anticonvulsant activity.

FIG. 2



Influence of chronic treatment with Δ^9-THC on the percentage of rats protected against hind-limb extension in the MES. Δ^9-THC dose-effect curves were obtained with the MES test after 7 days of Δ^9-THC (5 mg/kg/day) or vehicle treatment. Data from animals acutely treated with Δ^9-THC are illustrated by unfilled circles; chronically treated, by filled circles. Curves were fitted by the method of Litchfield and Wilcoxon (17). Each point represents results from 12 rats. MES test conducted at an ambient temp. of 22°C.

Effect of various cannabinoids on the MES test. The data in Table IV indicate that all the cannabinoids investigated exhibit anticonvulsant activity against MES. There are, however, marked differences in potency among the drugs. For example, a test for relative potency (17) indicated that the metabolite of Δ^9-THC, 11-hydroxy-Δ^9-THC, is more active in mice than the parent compound ($P \leq 0.05$). In contrast to the mouse data, Δ^9-THC and its 11-hydroxy metabolite have similar potencies

TABLE IV

Effects of Cannabinoids in Maximal Electroshock and Bar-walk Tests*

Drug	Species	Peak-effect time (hr)**	ED50 (mg/kg)	TD50 (mg/kg)	Protective Index (TD50/ED50)
Δ^9-THC	mouse	2	101 ± 26†	84 ± 28†	0.8 ± 0.07†
Cannabidiol	mouse	1	118 ± 15†	175 ± 6.8†	1.5 ± 0.1†
DMHP‡	mouse	2	13 ± 3.8†	5.4 ± 3.0†	0.4 ± 0.1†
d, ℓ-threo-DMHP	mouse	2	6.8 (5.4-8.5)‡	---	---
d, ℓ-erythro-DMHP	mouse	2	24 (18-32)‡	---	---
11-OH-Δ^9-THC	mouse	1	14 (11-16)‡	22 (17-27)‡	1.6
Cannabinol	mouse	1	230 (215-246)‡	230 (219-242)‡	1.0
Δ^8-THC	mouse	2	83 (61-113)‡	152 (101-228)‡	1.8
Δ^9-THC	rat	1	4.3 (3.9-4.8)‡	5.0 (3.5-7.3)‡	1.2
11-OH-Δ^9-THC	rat	0.25	3.4 (2.5-4.7)‡	---	---

* All experiments were performed at ambient temp. of approximately 22°C.

** The peak-effect times for the anticonvulsant and toxic effects were similar.

† Mean and S. D. determined from results of three experiments; mean ED50 for Δ^9-THC is not statistically different from mean ED50 for cannabidiol ($P > 0.05$), but both are statistically different from ED50 for DMHP ($P < 0.05$); mean TD50 values are statistically different from each other ($P < 0.05$); mean protective indices are statistically different from each other ($P < 0.05$); as indicated by analysis of variance and the Newman-Kuels multiple means test (18).

‡ DMHP, dimethylheptylpyran.

‡ Median-effective doses and their 95% confidence limits determined from results of one experiment.

in rats ($P > 0.05$) (17). In mice dimethylheptylpyran (DMHP) is approximately 8 times more potent than Δ^9-THC; DMHP, on the other hand, appears to be about as potent as diphenylhydantoin (DPH). In three DPH experiments with mice, the mean ED50 value and its S. D. were 9.2 ± 1.7 mg/kg; such figures compare favorably with the DMHP values shown in Table IV.

There are also marked differences in peak-effect times among the cannabinoids (Table IV). In both mice and rats the 11-hydroxy derivative has an earlier peak-effect time than does Δ^9-THC.

Relation between motor toxicity and anticonvulsant activity (protective index). In addition to the ED50s, the TD50 (bar-walk) values and the protective indices (TD50/ED50) are shown in Table IV. The numbers for the first three drugs listed in the Table, Δ^9-THC, cannabidiol (CBD) and DMHP, represent the means and standard deviations of three experiments with each drug. The remaining data are the results of one experiment for each of the agents listed. As shown in the Table, the protective indices range from 0.4 for DMHP to 1.8 for Δ^9-THC. A statistical comparison between the values for Δ^9-THC, CBD and DMHP was made, and the results indicate that these three protective indices are significantly different from one another ($P < 0.05$).

Discussion

Because hypothermia per se can alter excitability of the central nervous system in mice (19) and Δ^9-THC in an anticonvulsant dose lowers body temperature (Table I), the question arises whether the anticonvulsant properties stem from a direct action on the nervous system, or are an indirect effect of hypothermia. Inasmuch as hypothermia can be

greatly minimized at an ambient temperature of 30°C (Table I), the anticonvulsant properties of Δ^9-THC were investigated at both 22° and 30°C in order to assess the role of hypothermia in determining the drug's effects.

The studies of Δ^9-THC's influence on various thresholds show that, of the four tests used, only the results of the 60-Hz-EST were affected by hypothermia (Table II, Fig. 1): In the absence of any marked hypothermia, Δ^9-THC exerted no effect on the 60-Hz-EST; but in the presence of hypothermia, this threshold was significantly lowered. These findings suggest that the hypothermic effect of Δ^9-THC is responsible for lowering the 60-Hz-EST; but such an explanation may be an oversimplification, since Brown (19) reported that hypothermia elevates rather than decreases the 60-Hz-EST in mice.

A parallel evaluation of the role of hypothermia in the MES response indicates that hypothermia does not appear to influence Δ^9-THC's effect (Table III). If the two sets of data, threshold and MES, are taken together, they imply that the anticonvulsant properties of Δ^9-THC proceed from a direct action on the central nervous system, rather than from an indirect action mediated by the drug's hypothermic activity.

In previously published reports, Δ^9-THC's anticonvulsant properties have been compared to DPH's (1, 11), and the present investigation in part confirms their similarities: Thus, Δ^9-THC, like DPH, abolishes hind-limb extension in the MES test and raises both the MES threshold and the 6-Hz-EST. Nevertheless, these two drugs are quite dissimilar with respect to their effect on PTZ-caused minimal seizures: Unlike Δ^9-THC, an ED50 of DPH, as determined against MES, does not enhance minimal seizures produced by PTZ (20). In very high doses (>100 mg/kg

i.p.), however, even DPH can exacerbate PTZ-induced minimal seizures (21).

Our experiments demonstrated that in rats a rapid and marked tolerance to Δ^9-THC develops in the MES test; tolerance has also been seen in the rat in kindled seizures (22); Killam and Killam (23) described the development of tolerance to Δ^9-THC in its ability to protect against photomyoclonic seizures in the baboon; furthermore, mice, too, develop tolerance to Δ^9-THC in the MES test (24).

In some species the mechanism of tolerance does not appear to involve any obvious dispositional or metabolic changes: In the rat we found that blood and brain levels of Δ^9-THC and its 11-hydroxy metabolite are not altered by the development of tolerance (25), while McMillan and his co-workers similarly failed to detect any difference in blood concentration of Δ^9-THC in tolerant and nontolerant pigeons (26). Our rat studies additionally showed that tolerance develops with repeated administrations, despite an accumulation of Δ^9-THC and its metabolites in the brain. In view of the results of our brain determinations of Δ^9-THC and its metabolites, in rats at least, tolerance to the protective effect in the MES test appears to be a functional adaptation of the central nervous system.

Our survey of several cannabinoids for anticonvulsant activity against the MES (Table IV) demonstrated that all those tested can block hind-limb extension. In the mouse the potencies varied greatly: from DMHP, the most active, to cannabinol, the least active. The DMHP findings support previously published indications that it is a relatively potent cannabinoid (1, 13); however, results of human studies reporting

its very high potency relative to DPH as an antiepileptic (13) have not been borne out in the present data because the two drugs have similar activity in mice.

Moreover, in mice 11-hydroxy-Δ^9-THC is several times more potent than its parent compound, a finding which is consistent with the relative activity of these two substances in producing other effects (27, 28, 29). No such difference, however, was observed in rats. On the other hand, in both species the 11-hydroxy metabolite has an earlier peak-effect time than does Δ^9-THC. This earlier peak-effect time might be explained if the metabolite is the active form of the drug, as has been suggested by some investigators (30); other explanations, however, are equally possible: For example, variations in rates of absorption from the site of administration or in rates of penetration into the central nervous system may also account for the observed differences in peak-effect time.

It is clear that the anticonvulsant effect and motor toxicity of the cannabinoids are at least partially separable, as is evidenced by the disparities in their protective indices. Of the three compounds most rigorously investigated, Δ^9-THC, DMHP and CBD, the latter exhibits the highest protective index, hence the lowest relative toxicity. Although in mice the protective index for CBD is markedly lower than the value of 7 for DPH, it compares favorably with the protective indices of about 1 to 2 for trimethadione and phenobarbital (15). Of greater significance is CBD's lack of marihuana-like psychic activity (10, 31); furthermore, very high doses of intravenously infused CBD do not produce in humans either the cardiac acceleration or the psychic effects that are characteristic

of even small doses of Δ^9-THC (32). When the absence of such activity is taken together with CBD's anticonvulsant characteristics in animals, then it becomes logical to suggest that the drug may warrant clinical evaluation as an antiepileptic agent.

Acknowledgements

The authors are especially indebted to Dr. Monique C. Braude of the Biomedical Research Branch, Divison of Research, NIDA, for providing the cannabis derivatives as well as 11-hydroxy-Δ^9-THC, and to Dr. John F. Howes of Sharps Associates for DMHP and its isomers. Thanks are due to Dr. Dixon M. Woodbury for his advice and encouragement and to Dr. Ewart A. Swinyard for his suggestions relating to the manuscript. The work was supported by USPHS pharmacology training grant GM-00153, USPHS program project grant 5P01-NS-04553, USPHS research grant DA-346-01A1, and a research grant from the Epilepsy Foundation of America.

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