Pharmacology Biochemistry & Behavior, Vol. 3, pp. 849–853.

Marihuana-like Activity of New Synthetic Tetrahydrocannabinols

BILLY R. MARTIN, WILLIAM L. DEWEY, LOUIS S. HARRIS, JACQUELINE BECKNER

Department of Pharmacology, Medical College of Virginia
Virginia Commonwealth University, Richmond VA 23298

AND

RAYMOND S. WILSON AND EVERETTE L. MAY

Laboratory of Chemistry, National Institute of Arthritis, Metabolic and Digestive Diseases
Bethesda MD 20014.

(Received 18 December 1974)

MARTIN, B. R., W. L. DEWEY, L. S. HARRIS, J. S. BECKNER, R. S. WILSON AND E. L. MAY. *Marihuana-like activity of new synthetic tetrahydrocannabinols.* PHARMAC. BIOCHEM. BEHAV. 3(5) 849–853, 1975. – 11-Methyl-Δ^8-, 9-nor-Δ^8-, and 9-nor-Δ^9-tetrahydrocannabinol (THC), newly synthesized cannabinoids which are not 11-hydroxyated in vivo, were tested for cannabinoid activity. Δ^8-,Δ^9-THC and each synthetic analog produced static ataxia in unanesthetized dogs, hypotension and bradycardia in anesthetized dogs, and decreased spontaneous activity in mice. All synthetic analogs tested produced a greater degree of tolerance to the behavioral effect in dogs than did Δ^8-THC. 11-Methyl-Δ^8-THC was more effective than Δ^8-THC in decreasing spontaneous activity in mice, but was less active in producing the behavioral and cardiovascular effects in dogs. 9-nor-Δ^9-THC was less active than Δ^9-THC, but 9-nor-Δ^8-THC was as active as Δ^8-THC in all observations. These results suggest that the 11-hydroxy metabolites of Δ^8- and Δ^9-THC are not solely responsible for the biological activity of tetrahydrocannabinols.

11-hydroxy-Δ^9-THC 11-hydroxy-Δ^8-THC 9-nor-Δ^9-THC 9-nor-Δ^8-THC Metabolism of THC Tolerance

A number of investigators have shown that Δ^8 and Δ^9-THC are the psychoactive constituents of marihuana. Moreover, it has been postulated [12, 17, 19] that the 11-hydroxy metabolites of Δ^8 and Δ^9-THC are largely responsible for their pharmacological activity.

Lemberger [13] and Perez-Reyes [17] independently administered Δ^9-THC to man and reported the rapid occurrence of 11-hydroxy-Δ^9-THC in plasma. The appearance of the 11-hydroxy metabolite in plasma was temporally related to the psychological effects experienced by each subject. It also has been reported that intravenous administration of 11-hydroxy-Δ^9-THC, like Δ^8 and Δ^9-THC, resulted in tachycardia and an intense psychological "high" [11, 12, 18]. In mice, 11-hydroxy-Δ^9-THC was shown to be more active than Δ^9-THC as measured by decreased spontaneous activity and an increased responsiveness to tactile and auditory stimuli [1]. In addition, Truitt [19] reported that Δ^8-THC was converted to the 11-hydroxy metabolite and that 11-hydroxy-Δ^8-THC was more active than Δ^8- or Δ^9-THC when administered to rats. These data suggest that Δ^8- and Δ^9-THC may owe a majority, or all, of their activity to the 11-hydroxy metabolites. One approach to testing this hypothesis was to determine whether cannabinoid activity existed in synthetic THC analogs that are neither hydroxylated at position eleven nor are metabolized to 11-OH derivatives in vivo.

We have recently synthesized two new analogs of Δ^8-THC [21] 9-nor-Δ^8-THC and 11-methyl-Δ^8-THC; and one new analog of Δ^9-THC, 9-nor-Δ^9-THC; (Wilson and May, submitted for publication), the structures of which are presented in Fig. 1. The methyl group at position 11 of 11-methyl-Δ^8-THC probably hinders conversion to the 11-hydroxy analog. Absence of the 11 position in 9-nor-Δ^8-THC and 9-nor-Δ^9-THC makes 11-hydroxylation impossible.

METHOD

Cannabinoids produced static ataxia and other characteristic behavioral changes in dogs which were semiquantitated by the rating scale presented in Table 1. The maximum effect on behavior was seen within 30 min after an IV injection of the drug. Three observers independently rated the behavior of each dog, and the mean of their scores was recorded. For each dog, the independent scores never differed by more than one rank.

Animals and Procedure

Cardiovascular experiments were conducted with mongrel dogs of either sex weighing between 8 and 12 kg and responses were recorded on a Grass Model 5 polygraph. The

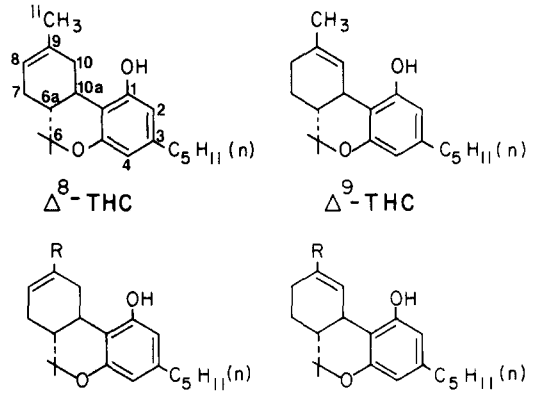

FIG. 1. Structures of Δ^8- and Δ^9-tetrahydrocannabinol (THC) and their newly synthesized analogs and important metabolites.

dogs were anesthetized with sodium pentobarbital (30 mg/kg, IV), and the left femoral vein was cannulated to allow intravenous administration of drugs. Arterial pressure was recorded from the left femoral artery through a cannula connected to a Statham pressure transducer. Mean arterial pressure was calculated by summing the diastolic pressure and one-third of the difference between systolic and diastolic pressures. Heart rate was obtained from an EKG recording using fine needle electrodes inserted through the skin. The THC vehicle was administered to all animals, before administration of the cannabinoid. Animals were given only one dose of one cannabinoid due to the drug's long duration of action. The maximum percent change in mean arterial blood pressure and heart rate (usually 30 min after the injection) was determined for each animal.

Albino mice (Swiss-Webster, Dublin Farms, 20–25 g) were used to examine the effects of these cannabinoids on spontaneous activity. A pair of mice were placed in each photocell activity chamber 10 or 90 min after the IP injection of vehicle or drug. Activity levels were accumulated for the next 15 min. Uncorrelated sample means were compared statistically by applying Student *t* tests.

The tail-flick method of D'Amour and Smith [3], as modified by Dewey *et al.* [4,5], was used to determine the analgesic activity of the cannabinoids in mice. Analgesic response was calculated by the method of Harris and Pierson [10] and expressed as percent maximum possible effect (percent MPE).

The phenylquinone writhing test of Pearl and Harris [16] was used in the present experiments. In mice, an SC injection of the cannabinoid was followed by an IP injection of *p*-phenylquinone (2 mg/kg) 10 min later. The number of squirms observed within a 1 min period at 1/3, 1, 4, and 8 hr after injection of *p*-phenylquinone was recorded.

The effect of the cannabinoids on barbiturate-induced sleeping time was measured in mice. Sodium hexobarbital (100 mg/kg) was injected IP at 1/3, 1, 4, and 8 hr after the IP injection of vehicle or cannabinoid. Mice were used only once. The sleeping time was the time between loss and regaining of the righting reflex.

Dogs were given tritium-labeled Δ^9-THC (35 μCi/0.5 mg Δ^9-THC/kg) intravenously, and were sacrificed 30 min after injection. In order to identify Δ^9-THC metabolites, plasma and brain homogenates were extracted with petroleum ether and subsequently with diethyl ether. These extracts were spotted on precoated thin-layer plates and developed in chloroform:acetone (1:1) for 90min. Areas corresponding to Δ^9-THC and its metabolites were scraped into liquid scintillation vials and counted for radioactivity.

The vehicle for all cannabinoids was a mixture of Emulphor (EL-620), ethanol, and saline [2]. One hundred mg of drug was dissolved in one ml of a 1:1 mixture of Emulphor and ethanol. Proper dilutions were made with saline.

TABLE 1

QUANTIFICATION OF THE BEHAVIORAL EFFECTS PRODUCED BY CANNABINOIDS*

Score	Behavioral Effects
0	no effect
1	slight depression of activity, slight static ataxia seen only after dog has been standing in one position for 3–5 min
2	walks with a prance-like placement of feet, exaggerated reflex to a swinging hand, and static ataxia after standing in one position for 2–3 min
3	tail is often tucked, some loss of tone in hind legs as evidenced by a semi-squatting position, static ataxia more pronounced and seen after dog stands in one position for 1–2 min, and nodding may be observed 30–60 min after injection
4	marked static ataxia, sways forward and backward and/or side to side, and almost falls after standing in one position for 1 min
5	cannot stand for longer than 30 sec without almost falling and frequently plunges about
6	lies prostrate on the floor

*Based upon a slight modification of the rating scales described by Walton *et al.* [20] and Dewey *et al.* [6]

TABLE 2

MAXIMUM BEHAVIORAL EFFECTS OF CANNABINOIDS AND THEIR ANALOGS ON OVERT BEHAVIOR IN DOGS*

Acute†

Dose (mg/kg)	Δ^9-THC	9-*nor*-Δ^9-THC	Δ^8-THC	9-*nor*-Δ^8-THC	11-Methyl-Δ^8-THC
0.1	0 (1)§		0 (1)		
0.2	3.3 (2)	1.3 (3)	0 (1)	0.6 (2)	0 (1)
0.4	4 (2)	2.3 (2)	2 (2)	1 (2)	0 (1)
0.8				2 (2)	0.6 (1)
1.0			5.3 (2)	4 (4)	1 (1)
1.6			4 (1)	5 (2)	
2.0					2.3 (2)
3.0					3.3 (2)

Chronic‡

Dose (mg/kg)	Δ^8-THC Day 1	Δ^8-THC Day 5	Δ^8-THC Percent Tolerance	9-*nor*-Δ^8-THC Day 1	9-*nor*-Δ^8-THC Day 5	9-*nor*-Δ^8-THC Percent Tolerance	11-Methyl-Δ^8-THC Day 1	11-Methyl-Δ^8-THC Day 5	11-Methyl-Δ^8-THC Percent Tolerance
1.0	5.3 (2)	3	47	3.6 (2)	13	70			
3.0							3.3 (2)	1	78

*Quantitated by the dog static-ataxia rating scale (Table 1).
†Acute animals received one intravenous injection.
‡Chronic animals received five daily intravenous injections.
§The mean score of all animals tested is presented with the number tested in parentheses.

RESULTS

The acute effects of Δ^8-THC, Δ^9-THC and their synthetic analogs on overt dog behavior (Table 1) are presented in Table 2. The data indicate that Δ^9-THC was more potent than 9-nor-Δ^9-THC whereas Δ^8-THC and 9-nor-Δ^8-THC were about equally active over the range of doses tested. 11-Methyl-Δ^8-THC exhibited difinite cannabinoid properties, although only at higher doses. Tolerance has been reported to develop to the behavioral effects of cannabinoids when administered chronically to dogs [6] and other laboratory animals [7,14]. Δ^8-, 9-nor-Δ^8-and 11-methyl-Δ^8-THC each were administered intravenously to 2 dogs daily for 5 consecutive days. By Day five, 47 percent tolerance developed to the behavioral effects of Δ^8-THC, 70 percent to 9-nor-Δ^8-THC, and 78 percent to 11-methyl-Δ^8-THC (Table 2).

Acute intravenous administration of these cannabinoids to anesthetized dogs produced decreases in heart rate and blood pressure which were maximal within 30 min and subsided after 1 to 2 hr. Due to a very limited supply of 9-nor-Δ^9-THC, only one dose (1.0 mg/kg) was given. The cardiovascular effects were similar to those observed in dogs receiving 1.0 mg/kg of Δ^9-THC. The dog receiving 9-nor-Δ^9-THC had a decrease in blood pressure of 31 percent and a decrease in heart rate of 42 percent, while the dogs receiving Δ^9-THC had a mean drop in blood pressure of 46 percent and a mean drop in heart rate of 39 percent. Three groups of 3 dogs received 2.4 mg/kg of either Δ^8-, 9-nor-Δ^8-, or 11 methyl-Δ^8-THC. Again, Δ^8-THC and 9-nor-Δ^8-THC were equipotent. Both produced a 44 percent decrease in heart rate; while Δ^8-THC caused a 52 percent decrease in blood pressure, and 9-nor-Δ^8-THC caused a 51 percent decrease in blood pressure. 11-Methyl-Δ^8-THC was much less active, producing only a 9 percent decrease in heart rate and no change in blood pressure.

The data presented in Table 3 show that there was a significant reduction in spontaneous activity of mice 10 minutes after they had received an acute IP injection of 10 or 20 mg/kg of Δ^8-THC and each of the synthetic THC analogs. Both Δ^8- and Δ^9-THC produced significant hypoactivity at 90 min after injection. These rodent studies showed that 9-nor-Δ^8-, 9-nor-Δ^9-, Δ^8-and Δ^9-THC had

TABLE 3

EFFECT OF ANALOGS ON SPONTANEOUS ACTIVITY OF MICE*

Drug	N	10 mg/kg†	N	20 mg/kg†	N	10 mg/kg‡
Vehicle	21	160 ± 13	22	145 ± 9	12	181 ± 22
Δ^9-THC	16	111 ± 23	11	148 ± 23	6	87 ± 31§
Δ^8-THC	10	95 ± 15[a]	11	90 ± 13[a]	6	28 ± 6[b]
9-*nor*-Δ^8-THC	11	117 ± 16§	11	90 ± 15[a]		
9-*nor*-Δ^9-THC	6	86 ± 11[b]	6	88 ± 24§		
11-methyl-Δ^8-THC	10	57 ± 9[b]	11	54 ± 9[b]		

*Number of interruptions of photocell recorded for 15 min (mean ± S.E.)
†Animals placed in activity chamber 10 min after injection and interruptions accumulated for 15 min
‡Animals placed in activity cages 90 min after injection and interruptions accumulated for 15 min
§Significantly different from vehicle by Student *t*-test ($p<0.05$)
[a]Significantly different from vehicle by Student *t*-test ($p<0.01$)
[b]Significantly different from vehicle by Student *t*-test ($p<0.001$)

similar effects on the activity of mice, and that 11-methyl-Δ^8-THC was somewhat more potent. Since Δ^8, and Δ^9THC were less active 10 min after injection apparently, the synthetic analogs had a quicker onset of action compared with Δ^8 or Δ^9THC. Mice were injected IP with 1 or 10 mg/kg, of Δ^8-, Δ^9THC or one of the analogs and were tested at 20 min, 1, 4, and 8 hr in the tailflick, hot-plate, phenylquinone writhing and hexobarbital potentiation tests. Mice were tested only once. There were no significant differences between control and test measures for any test at any time.

Dogs were injected with ^{3}H-Δ^9-THC and sacrificed at the time of maximum behavioral effects. Brain homogenate and plasma were extracted with petroleum ether and diethyl ether, and resultant extracts were examined by thin-layer chromatography. At the time of peak behavioral activity, 46 percent of the extractable radioactivity in brain was due to unchanged Δ^9-THC and only 10 percent was due to 11-OH-Δ^9-THC. Further, a somewhat similar ratio of Δ^9-THC to 11-OH-Δ^9-THC was observed in plasma. These data agreed favorably with the mouse brain concentration of Δ^9-THC and of 11-OH-Δ^9-THC at the time of peak behavioral activity as reported by Gill *et al.* [8].

DISCUSSION

It is noteworthy that compounds structurally related to the cannabinoids which cannot be converted to 11-hydroxy metabolites have previously been examined [4]. Some of these compounds exhibit pharmacological properties which may be similar to the tetrahydrocannabinols. However, the structures of all these compounds are sufficiently different from the tetrahydrocannabinols to preclude an assumption of a common mechanism or site of action. Herein lies the significance of the compounds examined in the present research, i.e., the only deviation from the natural tetrahydrocannabinol structure involved removal of the 11-methyl (9-nor-Δ^9-THC and 9-nor-Δ^8-THC) or the addition of methyl at the 11-position (11-methyl-Δ^8-THC). Presumably these latter compounds are structurally similar enough to Δ^9- and Δ^8-THC to have similar properties of distribution and receptor occupation. All of the normal routes of in vivo tetrahydrocannabinol metabolism not involving the 11-position would appear to be available to these compounds since the naturally occuring Δ^8 or Δ^9 double bond in these synthetic analogs is retained. Virtually all known metabolic transformation of the cyclohexene ring occur at the double bond itself [9] or at position allylic to the double bond: positions 11 and 7 in Δ^8-THC and positions 11 and 8 in Δ^9-THC [1].

All of the synthetic analogs in this study produced hypoactivity in mice similar to that caused by Δ^8 and Δ^9-THC. However, although data is not included, it appeared that all the synthetic compounds had a shorter onset of action than did Δ^9-THC. The analogs had similar behavioral and cardiovascular properties when administered to dogs, even though 11-methyl-Δ^8-THC was much less active. Since 11-methyl-Δ^8-THC was very effective in producing hypoactivity in mice, but had little effect on the cardiovascular system of the dog, it was obvious that more than one paradigm was necessary in order to establish cannabinoid activity. The pharmacological activity of these synthetic analogs clearly indicates that 11-hydroxylation was not a prerequisite for cannabinoid activity.

An interesting question was raised as to why a greater degree of tolerance developed to the behavioral effects of 9-nor-Δ^8-THC and 11-methyl-Δ^8-THC than to Δ^8-THC. One possible explanation is that the active form of the two synthetic compounds may persist for a longer period than that for Δ^8-THC in dogs. This is possible because Δ^8-THC

is known to be metabolized to the inactive 9-carboxy-Δ^8-THC [15]. Therefore, 9-nor-Δ^8-THC and 11-methyl-Δ^8-THC may persist longer in an active form in the body than Δ^8-THC because matabolic deactivation by conversion to 9-carboxy compounds may not be available to them.

In related experiments, dogs were given radiolabeled Δ^9-THC and were sacrificed at the time of peak behavioral activity. The major portion of the radioactivity in brain and plasma corresponded to unchanged Δ^9-THC with the remainder divided among 11-OH-Δ^9-THC, 8β-OH-Δ^9-THC, 8α-OH-Δ^9-THC, 8, 11-DiOH-Δ^9-THC, and more polar metabolites. Probably very little of the behavioral activity was due to the 8-OH-Δ^9-THC metabolites since it has been demonstrated that they are much less active in state dependent studies (unpublished results) and in producing psychological "highs" in man [18]. These data indicate that Δ^9-THC itself may be active since it was present in high concentrations in brain when pharmacological effects were at a maximum.

The results of the present investigations show that cannabinoids which can not be converted to an 11-hydroxy metabolite have similar pharmacologic profiles to Δ^8- and Δ^9-THC in mice and dogs. However, these data do not preclude the possibility that other metabolites of these newly synthesized cannabinoids may exhibit some behavioral activity. It appears that cannabinoid activity of Δ^8-, Δ^9-THC can be due to their parent compound even though some of their metabolites also have cannabinoid activity.

ACKNOWLEDGEMENTS

Emulphor EL-620 was kindly supplied by General Aniline and Film Corporation. The research was supported by USPHS Grant Number DA-00490.

REFERENCES

1. Christensen, H. D., R. I. Freudenthal, J. T. Gidley, R. Rosenfeld, G. Boegli, L. Testino, D. R. Brine, C. G. Pitt and M. E. Wall. Activity of Δ^8- and Δ^9-tetrahydrocannabinol and related compounds in the mouse. *Science* **172:** 165–167, 1971.
2. Cradock, J. C., J. P. Davignon, C. L. Sitterst, and A. M. Guarino. An intravenous formulation of Δ^9-tetrahydrocannabinol using a nonionic surfactant. *J. Pharm. Pharmac.* **25:** 345, 1973.
3. D'Amour, F. E. and D. L. Smith. A method for determining loss of pain sensation. *J. Pharmac. exp. Ther.* **72:** 74–79, 1941.
4. Dewey, W. L., L. S. Harris, J. F. Howes, J. S. Kennedy, F. E. Granchelli, H. G. Pars and R. K. Razdan. Pharmacology of some marihuna constituents and two heterocyclic analogues. *Nature* **266:** 1265–1267, 1970.
5. Dewey, W. L., L. S. Harris, J. F. Howes and J. A. Nuite. The effect of neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. *J. Pharmac. exp. Ther.* **175:** 432–442, 1970.
6. Dewey, W. L., J. Jenkins, O'Rourke, and L. S. Harris. The effects of chronic administration of trans-Δ^9-tetrahydrocannabinol on behavior and the cardiovascular system of dogs. *Archs int. Pharmacodyn. Ther.* **198:** 118–131, 1972.
7. Dewey, W. L., D. E. McMillan, L. S. Harris and R. F. Turk. Distribution of radioactivity in brain of tolerant and nontolerant pigeons treated with ^{3}H-Δ^9-tetrahydrocannabinol. *Biochem. Pharmac.* **22:** 399–405, 1973.
8. Gill, E. W. and G. Jones. Brain levels of Δ^1-tetrahydrocannabinol and its metabolites in mice--correlation with behavior, and the effect of the metabolic inhibitors SKF-525A and piperonyl butoxide. *Biochem. Pharmac.* **21:** 2237–2248, 1972.
9. Gurny, O., D. E. Maynard, R. G. Pitcher and R. W. Kierstead. Metabolism of (-)-Δ^9- and (-)-Δ^8-tetrahydrocannabinol by monkey liver. *J. Am. Chem. Soc.* **94:** 7928–7929, 1972.
10. Harris, L. S. and A. K. Pierson. Some narcotic antagonists in the benzomorphan series. *J. Pharmac. exp. Ther.* **143:** 141–148, 1964.
11. Hollister, L. E. Structure-activity relationships in man of cannabis constituents, and homologs and metabolites of Δ^9-tetrahydrocannabinol. *Pharmacologia* **11:** 3–11, 1971.
12. Lemberger, L., R. E. Crabtree and H. M. Rowe. 11-Hydroxy-Δ^9-tetrahydrocannibinol: Pharmacology, disposition, and metabolism of a major metabolite in man. *Science* **177:** 62–64, 1972.
13. Lemberger, L., N. R. Tamarkin, J. Axelrod and I. J. Kopin. Delta-9-tetrahydrocannabinol: Metabolism and disposition in long-term marihuana smokers. *Science* **173:** 72–74, 1971.
14. McMillan, D. E., W. L. Dewey and L. S. Harris. Characteristics of tetrahydrocannabinol tolerance. *Ann. N.Y. Acad. Sci.* **191:** 83–99, 1971.
15. Mechoulam, R., Z. Ben-Zvi, S. Agurell, I. M. Nilsson, J. L. G. Nilsson, H. Edery and Y. Grunfeld. Δ^6-tetrahydrocannabinol-7-oic acid, a urinary Δ^6-THC metabolite: Isolation and synthesis. *Experientia* **29:** 1193–1195, 1973.
16. Pearl, J. and L. S. Harris. Inhibition of writhing by narcotic antagonists. *J. Pharmac. exp. Ther.* **154:** 319–323, 1966.
17. Perez-Reyes, M. A. Lipton, M. C. Timmons, M. E. Wall, D. R. Brine and K. H. Davis. Pharmacology of orally administered Δ^9-tetrahydrocannabinol. *Clin. Pharmac. Ther.* **14:** 48–55, 1973.
18. Perez-Reyes, M., M. C. Timmons, M. A. Lipton, H. D. Christensen, K. H. Davis and M. E. Wall. A comparison of the pharmacological activity of Δ^9-tetrahydrocannabinol and its monohydroxylated metabolites in man. *Experientia* **29:** 1009–1010, 1973.
19. Truitt, E. B. Biological disposition of tetrahydrocannabinols. *Pharmac. Rev.* **23:** 273–278, 1971.
20. Walton, R. P., L. F. Martin, and J. H. Keller. The relative activity of various purified products obtained from American grown hashish. *J. Pharmac. exp. Ther.* **62:** 239–251, 1938.
21. Wilson, R. S. and E. L. May. 9-nor-Δ^8-THC, a cannabinoid of metabolic interest. *J. Med. Chem.* **17:** 475–576, 1974.