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Pharmacological Potency of R- and S-3′-Hydroxy-Δ^9-Tetrahydrocannabinol: Additional Structural Requirement for Cannabinoid Activity[1]

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MARTIN, B. R., M. J. KALLMAN, G. F. KAEMPF, L. S. HARRIS, W. L. DEWEY AND R. K. RAZDAN. *Pharmacological potency of R- and S-3′-hydroxy-Δ^9-tetrahydrocannabinol: Additional structural requirement for cannabinoid activity.* PHARMACOL BIOCHEM BEHAV 21(1) 61–65, 1984.—The pharmacological potency of R- and S-3′-hydroxy-Δ^9-tetrahydrocannabinol (THC) was compared to that of Δ^9-THC as well as R/S-3′-OH-Δ^9-THC. The S-isomer was found to be considerably more potent than the R-isomer in producing hypoactivity in mice, static-ataxia in dogs, and in generalization testing in rats trained to discriminate Δ^9-THC from vehicle. S-3′-OH-Δ^9-THC was more active than Δ^9-THC in these tests which means that Δ^9-THC may be either activated or inactivated *in vivo* depending upon which metabolite is formed. The difference in potency of these isomers suggests that the conformation of the side chain is critical for behavioral activity. The R and S isomers were found to be equally active in producing hypothermia in mice which is in contrast to the behavioral effects.

Cannabinoids 3′-Hydroxy-Δ^9-tetrahydrocannabinol Δ^9-Tetrahydrocannabinol Stereoisomers Ataxia Hypothermia Drug discrimination Behavioral Rats Mice Dogs

THE metabolism of the cannabinoids has been studied extensively in an effort to determine what contribution the metabolites make to the pharmacological effects of the parent compound. Hydroxylation of Δ^8-THC in the side chain (at positions 1′ and 3′) was first reported by Maynard *et al.* [11]. Hydroxylation in the 3′-position as well as at other side chain positions has been found in the perfused dog-lung preparation [17] and *in vitro* preparations of mouse [1, 7, 12] and monkey liver [16], to name a few. 3′-Hydroxylation has also been reported with *in vitro* preparations of human liver [4]. Interest in the side chain metabolites increased once it had been established that they had pharmacological activity similar to that of the parent compound. The 3′-hydroxylated metabolite is the most active of the side-chain metabolites in the Δ^8-THC series [1,12], and 3′-OH-Δ^9-THC is approximately 3 times more active than Δ^9-THC [5]. It has been difficult to establish the importance of side-chain hydroxylation. Despite its high potency, 3′-OH-Δ^9-THC may make only a modest contribution to Δ^9-THC's behavioral effects. Evidence for appreciable brain concentrations of 3′-OH-Δ^9-THC, as well as its time course, following Δ^9-THC administration is lacking. Trace quantities of 3′-OH-Δ^9-THC have been found in liver from several species treated with Δ^9-THC [7]. The low tissue concentrations of 3′-OH-Δ^9-THC may be due to the fact that it is extensively metabolized to more polar compounds [7].

Introduction of a hydroxyl group at position 3′ results in an asymmetric carbon center so that the two configurations depicted in Fig. 1 are possible. Synthesis of the R- and S-3′-OH isomers of Δ^9-THC have provided us with the opportunity to determine which of these isomers exhibits cannabinoid activity.

METHOD

Drugs

The cannabinoids were prepared for injection by dissolving 10 mg of drug in 100 μl of a 1:1 mixture of emulphor

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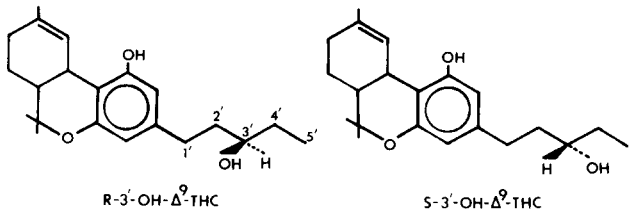

FIG. 1. Structures of R- and S-3′-OH-Δ^9-THC.

(GAF Corporation, Linden, NJ) and ethanol by sonication. Appropriate dilutions were made with a 1:1:18 mixture of emulphor:ethanol:saline.

The synthesis of the racemate has been described [5] and those of the isomers are to be published elsewhere. The preparation of these compounds results in approximately 10% of the material having the double bond in the 8-position and the remainder in the 9-position. The synthesis actually produced mixtures enriched with either R- or S-3′-OH-Δ^9-THC. Final resolution of these compounds resulted in S-3′-OH-Δ^9-THC that contained 9% of the R isomer and R-3′-OH-Δ^9-THC that contained 15% of the S isomer.

Measurement of Hypoactivity and Hypothermia in Mice

Male ICR mice (22–30 g) were housed in the laboratory for 24 hr before treatment. The ambient temperature of the laboratory, which varies from 21 to 24°C from day to day, was recorded at the beginning and end of each experiment. Rectal temperature was determined by a thermistor probe (inserted 25 mm) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) just prior to vehicle or drug administration. Following the initial temperature determinations, mice were injected intravenously with either vehicle or drug (0.1 ml/10 g of body weight) and immediately placed in photocell activity chambers (mice were not habituated to the apparatus). After the animals were placed in the chambers, interruptions of the photocell beams were recorded for 10 min. The results were expressed as percent of vehicle-treated mice, and the ED50's and their confidence limits were determined by the method of Litchfield and Wilcoxone [9]. The mice were removed from the activity chambers, and rectal temperatures were measured immediately and at 10-min intervals up to 60 min after drug administration. The difference between pre- and post-injection temperatures were calculated for each animal. These experiments were always carried out between 8 a.m. and 11 a.m.

Overt Behavioral Measurements in Dogs

The ability of compounds to produce static ataxia (animals sway forward and backward and/or side to side while standing in a fixed position) and other behavioral effects characteristic of cannabinoids was examined in mongrel dogs of either sex (10–15 kg). The animals were observed for their degree of spontaneous activity, gait, tail-tuck, etc., prior to drug administration. The animals were then injected intravenously with the cannabinoid or vehicle (1 ml/5 kg of body weight), and their behavior was rated at 5-min intervals according to a slight modification [10] of the Walton static-ataxia scale [15]. The rating scale ranges from 0 for no effect to 6 for maximum effect. Slight CNS depression and static ataxia were assigned a score of 1. Dogs that prance when they walk, exhibit exaggerated reflexes, and static ataxia after standing in one position for 2–3 min are given a score of 2. A static ataxia rating of 3 is given when the dogs stand in one position for 1–2 min and his tail is tucked. A score of 4 is given for marked static ataxia and 5 if the dog cannot stand for longer than 30 sec without staggering about. A score of 6 indicates the dog was prostate. At least three observers rated each animal and their scores were averaged. All observers were "blind" to the drug treatment.

THC Discrimination in Rats

Sprague-Dawley derived male rats (Dominion Labs, Dublin, VA), weighing 175 g at the initiation of discrimination training, were housed individually where illumination was cycled on a 12-hr light/dark schedule (light onset 0600), and temperature was controlled at 21±1°C, and relative humidity was 40–60%. Water was available in the home cage at all times while Purina lab chow was rationed to maintain the rats at approximately 85% of their free-feeding weights.

All drug discrimination training and testing was conducted in a standard 2-lever operant chamber (Coulbourne Inst., Titusville, MD, Model E-10-10). Chambers were fitted with 2 levers located at one end of the chamber and 3 cm above the floor of the chamber. A dipper, located between the two levers provided milk reinforcement (0.01 ml) subsequent to correct responding. The chamber was illuminated by a white houselight located 3 cm below the ceiling. Each operant chamber was housed in a sound-attenuated outer chamber to control extraneous noise. The delivery of reinforcers contingent on correct-lever responding under a fixed ratio (FR-20) schedule of reinforcement was controlled by a modified Rockwell Aim minicomputer (MicroInterfaces, Inc., Minneapolis, MN). The accumulation of total responses emitted on each lever and total reinforcers delivered were controlled by the minicomputer for each daily session for each rat.

All rats were initially trained to lever press for a diluted sweetened milk reinforcement when only one of the two levers was present in the chamber. Once lever pressing was established, both levers were placed in the chamber and discrimination training was initiated. Thirty min before each daily 15-min session, rats were injected IP with either 3 mg/kg Δ^9-THC or the Δ^9-THC vehicle (1 ml/kg). On any given day, half of the subjects were pretreated with the drug and the other half were pretreated with the vehicle. For half of the rats, responding on the left lever was reinforced when Δ^9-THC was given before the session and responding on the right lever was reinforced when the pretreatment was a vehicle injection. The opposite reinforced lever-drug pretreatment association existed for the other half of the rats. All rats were required to elicit a total of 32 responses on the correct lever (FR-32) before reinforcement was delivered. Drug treatments were alternated in a counterbalanced sequence across days (i.e., THC, THC, V, V, or V, V, THC, THC). Daily sessions were conducted Monday–Friday with the first 2.5 min of the second alternation session conducted under extinction conditions so that discriminability of the two cues (Δ^9-THC and vehicle) could be assessed. The remaining 12.5 min served as a daily training session when appropriate responding was reinforced.

Generalization testing was conducted after all rats reached a minimum criterion of drug-lever responding at 80% when Δ^9-THC was given presession and 20% when the vehicle was given presession. Testing of novel compounds occurred on Friday when rats were injected 30 min presession

TABLE 1

EFFECT OF Δ^9-THC AND ITS 3'-HYDROXYLATED ANALOGS ON BODY TEMPERATURE OF MICE*

Dose (mg/kg)	Δ^9-THC N	Δ^9-THC °C	R/S-3'-OH-Δ^9-THC N	R/S-3'-OH-Δ^9-THC °C	R-3'-OH-Δ^9-THC N	R-3'-OH-Δ^9-THC °C	S-3'-OH-Δ^9-THC N	S-3'-OH-Δ^9-THC °C
V	24	0.3 ± 0.3	30	0.7 ± 0.4	36	0.5 ± 0.7	36	0.5 ± 0.7
0.3			6	0.8 ± 0.3			6	1.3 ± 0.3
1.0	18	2.5 ± 0.2	6	1.1 ± 0.4	6	1.3 ± 0.4	6	2.9 ± 0.5
1.5	6	3.2 ± 0.2	12	3.2 ± 0.4	12	2.5 ± 0.2	12	3.0 ± 0.3
3.0	18	3.6 ± 0.4	12	3.2 ± 0.5	12	3.3 ± 0.5	12	3.6 ± 0.4
10.0	18	3.7 ± 0.5	6	4.0 ± 0.4	6	4.7 ± 0.6	6	3.3 ± 0.3

*Results are the differences betwen preinjection rectal temperatures and the lowest temperatures up to 60 min postinjection.

Means ± S.E. are presented. Vehicle (V). Numbers of animals per group (N).

and placed in operant chambers for 2.5 min with no reinforcement delivered during testing. Training sessions continued Monday–Thursday to maintain discrimination of the original training cues. For any given session, the total number of responses emitted on each lever and total reinforcers delivered were recorded. Both responses/sec and percent drug-lever responding were calculated for each rat for each session. Once testing was completed for all animals at a given test dose, the mean±S.E.M. of the session data was calculated for all of the rats. Test doses were given in a randomized sequence.

RESULTS

Hypoactivity and Hypothermia in Mice

The time course of cannabinoid-induced hypoactivity was determined by treating mice with Δ^9-THC (2.5 mg/kg IV) and measuring spontaneous activity at the indicated times. Mice were used only once. The interruptions of the photocell beam (mean±S.E., N=6) were 37±7, 39±29, 36±16, 23±7, 62±12, and 67±11 for time periods 0–10, 10–20, 20–30, 30–40, 50–60, and 110–120. Vehicle-treated mice produced 95±11 interruptions for the 0–10 period. Naive-treated mice produced behavioral activity similar to that for the vehicle-treated mice. The 0–10 min time period was chosen for measurement of hypoactivity which allowed for the subsequent measurement of the time course of hypothermia in the same animals.

Δ^9-THC, the racemate of 3'-OH-Δ^9-THC and the two individual isomers produced hypoactivity in mice in a dose-related manner. The ED_{50} (confidence limits, C.L.) for Δ^9-THC was 2.10 (1.30–3.30) mg/kg while that of R/S-3'-OH-Δ^9-THC was 0.85 (0.40–1.78) mg/kg. The ED_{50} (C.L.) for the S isomer was 0.34 (0.14–0.79) mg/kg which was considerably less than the 2.48 (1.16–5.31) mg/kg for the R isomer.

There appeared to be little difference in the potencies of Δ^9-THC, R/S-3'-OH-Δ^9-THC and its individual isomers regarding their hypothermic activity (Table 1). Analysis by Duncan's multirange test revealed no significant differences among the hypothermic effects at either 1.5, 3 or 10 mg/kg. At 1 mg/kg, the hypothermia produced by Δ^9-THC and S-3'-OH-Δ^9-THC was significantly higher than that produced by either R/S- or R-3'-OH-Δ^9-THC.

THC Discrimination in Rats

The results of substituting various doses of Δ^9-THC in animals trained to discriminate between 3 mg/kg Δ^9-THC and saline are presented in Fig. 2. A low dose of Δ^9-THC (1 mg/kg) produced saline-like responding, while 2 mg/kg produced intermediate responding. Higher doses (3 and 4 mg/kg) produced greater than 80% Δ^9-THC-like responding. As can be seen in Fig. 2, 11-OH-Δ^9-THC also produced generalization of the Δ^9-THC training cue. 11-OH-Δ^9-THC was found to be more potent than Δ^9-THC since a dose of 0.75 mg/kg produced generalization of the Δ^9-THC (3 mg/kg) training cue at a level of stimulus control approximately equivalent to that produced by the training cue alone. Neither Δ^9-THC nor 11-OH-Δ^9-THC significantly attenuated the response rates at the doses tested.

The results of generalization testing with R/S-3'-OH-Δ^9-THC and its isomers are presented in Fig. 3. The racemate produced cannabinoid discrimination effects with an ED_{50} (C.L.) of 0.31 (0.15–0.65) mg/kg. The racemate was approximately 5 times more active than Δ^9-THC since the latter had an ED_{50} (C.L.) of 1.5 (1.1–2.2) mg/kg. Testing with the R and S isomers indicated that the S isomer was the more active. The generalization curve observed for the S isomer, ED_{50} (C.L.) of 0.25 (0.12–0.49) mg/kg, overlapped that observed for the racemate. The R isomer also produced Δ^9-THC generalization, ED_{50} (C.L.) of 1.67 (1.12–2.50) mg/kg, but this compound produced these effects at a dose 7 times that required for the S isomer. While the racemate and the S and R isomers did produce Δ^9-THC-like stimulus control, significant response rate suppression was not observed at these doses.

Overt Behavioral Effects in Dogs

R/S-3'-OH-Δ^9-THC, the isomers and Δ^9-THC were evaluated in the dog static ataxia test at two doses in duplicate. Δ^9-THC, at doses of 0.1 and 0.2 mg/kg IV, produced average scores of 1 and 2, respectively. The racemate was more active than Δ^9-THC in this test since doses of 0.1 and 0.2 mg/kg resulted in scores of 1.5 and 3.5, respectively. As in the other tests, the S isomer was more potent than the R isomer. The S-3'-OH-Δ^9-THC produced scores of 1.5 at 0.1 mg/kg and 4.5 at 0.2 mg/kg. The R isomer produced a score

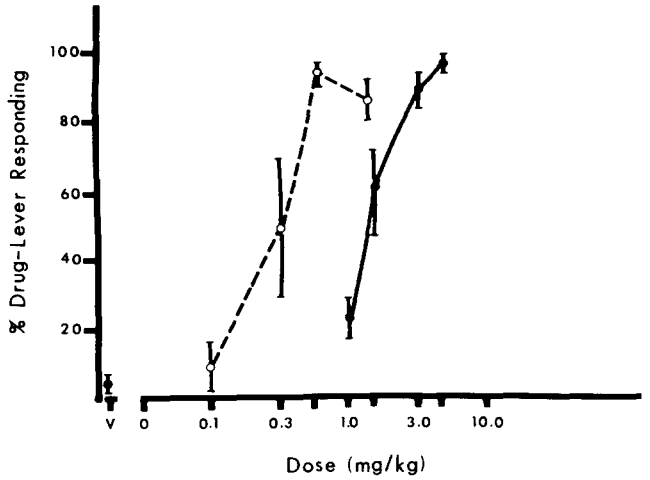

FIG. 2. The percent drug-lever responding produced by testing under extinction contingencies with various doses of the training drug (●—●) and with 11-OH-Δ^9-THC (○— —○). Each plotted point represents a mean±S.E.M. for 8 rats. Prior to testing, all of the rats were trained to discriminate 3 mg/kg Δ^9-THC from vehicle.

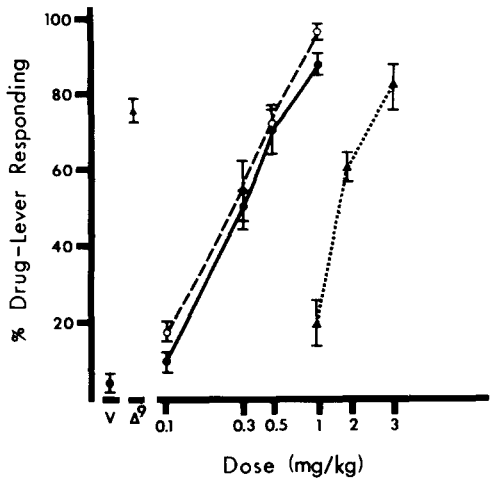

FIG. 3. The percent drug-lever responding produced by testing under extinction contingencies with various doses of R/S-3′-OH-Δ^9-THC (●—●) and the R (▲) and S (○) isomers. Each point represents a mean response for 8 rats±S.E.M. All animals were previously trained to discriminate between vehicle and Δ^9-THC (IP) injections (3 mg/kg) prior to testing.

of only 1.0 at 0.2 mg/kg and 4.0 at 5 mg/kg. The only major difference between Δ^9-THC and these analogs was the rapid onset of effect seen with the analogs. Frequently, the maximum behavioral effects of R/S-, R- and S-3′-OH-Δ^9-THC were seen within 5 min of administration, whereas Δ^9-THC produced maximal effects between 15 and 30 min after treatment. The duration of action was not appreciably different between Δ^9-THC and these 3′-hydroxylated derivatives.

DISCUSSION

The results presented herein are consistent with our previous observations that R/S-3′-OH-Δ^9-THC is more potent than Δ^9-THC [5]. We have extended those observations by analyzing R/S-3′-OH-Δ^9-THC in rats trained to discriminate between vehicle and Δ^9-THC. The racemate is indeed THC-like in this drug discrimination paradigm and is more potent than Δ^9-THC. The major aim of this study was to determine whether or not the geometrical isomers of 3′-OH-Δ^9-THC were equipotent in producing cannabinoid pharmacological effects. In all of the tests, with the exception of hypothermia, the S isomer was clearly more potent than the R isomer. R-3′-OH-Δ^9-THC was approximately 7 times less active than the S isomer in producing hypoactivity and in producing the Δ^9-THC-like discriminative cue and almost 5 times less active in the dog static-ataxia test when 0.2 mg/kg was administered. Calculation of exact potency ratios is complicated somewhat by the lack of absolute purity of the isomers. However, estimation of potency ratios can be made by taking the percentage of the impurity into consideration as has been done with other compounds, such as with the stereoisomers of nicotine [3] and Δ^9-THC [8]. Considering the fact that the R isomer contains a 15% impurity of the S isomer, the hypoactivity and THC-like discriminability of the R isomer preparation could be due almost entirely to the S isomer contamination. The subjective nature of the dog static-ataxia test makes it difficult to draw quantitative conclusions (this test is more ideally suited for qualitative than quantitative measurements). While it cannot be ruled out that the R isomer has some effect in the dog static-ataxia test, the S isomer contamination clearly contributes a significant amount to the activity of the R isomer preparation. In addition, the presence of a small amount of the R isomer in the S isomer preparation could lead to a slight underestimation of the potency of the S isomer since the R isomer is clearly much less active than the S isomer.

Although the S isomer is clearly much more active than the R isomer in producing behavioral effects, such was not the case with hypothermia. The isomers appeared to be equiactive in producing hypothermia and were approximately equiactive to Δ^9-THC. It may be that hypothermia is mediated through a mechanism different from that responsible for the other central effects. Other THC analogs have been synthesized that do not produce both hypothermia and hypoactivity which supports the notion of separate mechanisms of cannabinoid action [14].

The interest in THC analogs with side chain hydroxyl groups arose from the fact that they were formed metabolically and that they were pharmacologically active. However, the pharmacological activity was established for the racemates of these side-chain hydroxylated compounds rather than their geometrical isomers [1, 5, 12]. The extent to which hydroxylation at the 3′ position contributes to the behavioral activity of Δ^9-THC cannot be established until it is determined which isomer is formed metabolically.

The pharmacological selectivity of the R and S isomers of 3′-OH-Δ^9-THC may provide some insight into possible mechanisms of action for the cannabinoids. Several investigators have suggested that Δ^9-THC produces many of its effects by altering the synthesis of prostaglandins (see [13] for a recent review). It is noteworthy that the side-chain configuration of the S isomer is the same as that of the prostaglandin side chain. Prostaglandin-like activity in the S isomer but not in the R isomer would lend credence to this hypothesis. There are also suggestions that cannabinoids may interact with specific receptors to produce some of their pharmacological effects [2,6]. One could speculate that the

side-chain interacts with a lipophilic region of a receptor through hydrophobic bonding. Hydroxylation of the side-chain could lead to hydrogen bonding and greater affinity for the receptor. Since side-chain hydroxylation is not essential for cannabinoid activity, it may be that the configuration of the side-chain in R-3′-OH-Δ^9-THC disallowed interaction with the receptor whereas the configuration of the side-chain in the S isomer permits binding to the receptor.

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