Life Sciences, Vol. 29, pp. 565-574 Pergamon Pre
Printed in the U.S.A.

BEHAVIORAL COMPARISONS OF THE STEREOISOMERS OF TETRAHYDROCANNABINOLS[1]

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(Received in final form June 4, 1981)

Summary

The potencies of (-)-trans-Δ^9-THC, (+)-trans-Δ^9-THC, (+)-cis-Δ^9-THC, (-)-trans-Δ^8-THC and (+)-trans-Δ^8-THC were compared in several different species. (-)-trans-Δ^9-THC was 100 times more potent than (+)-trans-Δ^9-THC in depressing schedule-controlled responding in monkeys. The (+)-trans isomers were less effective than their corresponding (-)-trans isomers in the dog static-ataxia test, but potency ratios could not be determined due to a lack of dose-responsiveness of the (+)-trans isomers. However, it appeared that their potency differed by at least ten fold. The potency of (+)-cis-Δ^9-THC in the dog static-ataxia test was comparable to that of (+)-trans-Δ^9-THC. The hypothermia in mice produced by the (-) isomers of trans-Δ^9-THC and trans-Δ^8-THC were 9.1 and 30.4 times greater than that produced by their respective (+)-isomers. Also, the potency ratio of the (+)- and (-)-trans-Δ^9-THC was 5.6 as measured by depression of spontaneous activity in mice. The magnitude of the potency ratios of the THC stereoisomers is dependent upon the species and the pharmacological test used.

(-)-trans-Δ^9-Tetrahydrocannabinol (THC) is the principal active ingredient in marihuana (1). The isomers of (-)-trans-Δ^9-THC include: (+)-trans-Δ^9-, (-)-cis-Δ^9- and (+)-cis-Δ^9-THC. The corresponding isomers of Δ^8-THC also exist. There is considerable interest in pharmacological comparisons of stereoisomers since these data are useful in predicting whether or not a drug is exerting its effects through receptor mechanisms. There have been suggestions that cannabinoids produce their behavioral effects through both nonreceptor (2) and receptor mechanisms (3). There is some evidence pointing to stereospecificity of cannabinoids. Edery et al. (4) have reported that (-)-trans-Δ^9-THC is > 20 times more potent than (+)-trans-Δ^9-THC and that (-)-trans-Δ^8-THC is > 4 times more potent than (+)-trans-Δ^8-THC. Jones et al. (5), using the mouse ring test, found that (-)-trans-Δ^9-THC was 13 times more potent than (+)-trans-Δ^9-THC. In addition, Rosell et al. (6)

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[1]This work was supported in part by grants DA-00490 and DA-00574-07 from the National Institute on Drug Abuse.
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0024-3205/81/060565-10$02.00/0

reported that (-)-trans-11-OH-Δ^9-THC was greater than 20 times more potent than (+)-trans-11-OH-Δ^9-THC in inhibiting electrically-induced contractions of the guinea-pig ileum. Unfortunately, not all of the above experiments have been carried out using optically pure isomers. We have used stereospecific syntheses to prepare four optically-pure stereoisomers of THC, and we report herein their biological activity in several pharmacological test systems.

Methods

Drugs

(+)-cis-Δ^9-THC was synthesized as previously described by one of us (7). (+)-trans-Δ^9- and Δ^8-THC were synthesized from (+)-α-pinene {$[\alpha]_D^{22}$ + 47.1° (neat); > 98% pure, Aldrich Chemical Co.} according to the procedure of Mechoulam et al. (8). All of the isomers were greater than 98% optically pure (-)-trans-Δ^8- and Δ^9-THC were obtained from the National Institute on Drug Abuse. For biological experimentation, the cannabinoids (100 mg) were dissolved in 1 ml of emulphor-ethanol (1:1) by sonication and appropriate dilutions were made with saline.

Schedule-controlled behavior in monkeys

Two male adult squirrel monkeys (Saimiri sciureus, Santa Cruz, Bolivia, Primate Imports, Port Washington, N.Y.) with prior experimental and drug histories were used as subjects. Initial free feeding weights were 975 and 1060 g. Animals were maintained at approximately 85% of their free feeding weight throughout the duration of the study. Diet consisted of Purina Monkey Chow and a daily vitamin supplement. Animals were individually housed in an isolated room with a 12 hr light-dark cycle. Water was continuously available in the home cages but not during experimental sessions. During experimental sessions the animals were placed in a primate chair with a restraint stock about the waist (9). The chair was equipped with a stimulus light, a response lever and an automatic feeder that delivered 97 mg Noyes banana-flavored pellets into a trough. The animal and chair were placed in a light- and sound-attenuating isolation chamber during the experimental session with ventilation and sound masking provided by a fan. Solid state programming equipment, cummulative recorders and counters were located in an adjacent room.

The animals had previously been trained on a variable interval (VI) 60-sec schedule. Prior to the onset of the study, sessions were conducted for 3 weeks, 5 days per week in order to stabilize responding. The length of the session was gradually increased from 10 to 60 min. At the end of the initial 3 week period, experimental sessions were conducted 5 days a week for 1 hr at approximately the same time each day. Sessions were in a 5-day cycle with the data obtained on the 4th day used to determine baseline. The 5th day of the cycle was the drug test day. Doses were given in either ascending or descending concentrations with each animal receiving each dose of both drugs one time. Vehicle injections were also administered to each subject. Injections were given i.m. in a volume of 1 ml/kg 30 min. prior to the start of the session. One subject was tested with the (-)-trans isomer first, the other was tested with the (+)-trans isomer first. In addition, each isomer was given in ascending doses to one monkey and descending doses to the other animal.

Overt behavior of dogs

Mongrel dogs of either sex, weighing 10-20 kg, were observed for five min. for degree of spontaneous activity, gait, tail-tuck, etc. The animals were then injected i.v. with the cannabinoid and their behavior rated according to the Walton (10) static-ataxia scale (Table 1). A typical test session consisted of five animals that received either vehicle, 0.2 mg/kg of (-)-trans-Δ^9-THC, or one of three other cannabinoid treatments. Animals were used only once. Behavior was scored by three observers who were blind with regard to treatment.

Table 1

Quantification of the Behavioral Effects Produced by Cannabinoids[a]

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.

[a]Based upon modification of the rating scale described by Walton et al. (10).

Body temperature of mice

Male ICR mice (22-30 g) were housed in the laboratory for 24 hr before treatment. The ambient temperature of the laboratory varied from 21 to 24°

from day to day. Rectal temperature was determined by a thermistor probe (inserted 25 mm) and a telethermometer (Yellow Springs Instrument Co., Yellow Spring, Ohio). Readings were taken just prior to and 10, 20, 40, 60 and 120 min after i.v. administration of either vehicle or test drug.

Spontaneous activity in mice

Mice were injected i.v. with either vehicle or test drug and 5 min. later a pair of mice from each group were placed in a photocell activity chamber (20 x 33 cm). Pairs of vehicle-treated mice were placed in two chambers which were run concurrently with pairs of THC-treated mice in four other chambers. After the animals were placed in the chambers, interruptions of the photocell beams were recorded for the time intervals of 0-10, 10-20, 20-30, 30-40, 40-50 and 50-60 min. The results were expressed as percent of control and the ED50's and their confidence limits were determined by the method of Litchfield and Wilcoxon (11).

Results

Schedule-controlled behavior in monkeys

The results for each subject are presented separately. Figure 1 shows the effects of (-)- and (+)-trans-Δ^9-THC on the rate of reinforcement in both subjects. Vehicle (V) had no effect in either subject. (-)-trans-Δ^9-THC produced a dose-dependent decrease in the rate of reinforcement. At doses of 1 mg/kg and greater, almost complete suppression of behavior was seen in both subjects. (+)-trans-Δ^9-THC, on the other hand, was inactive up to 30 mg/kg in both subjects on this measure.

Figure 2 represents the effects on response rate. Again, vehicle had no effect in either subject. (-)-trans-Δ^9-THC produced a dose-dependent decrease in responding. The minimal reliably effective dose in each subject was 0.3 mg/kg and almost complete suppression of responding was seen at a dose of 1.0 mg/kg and higher. At these doses observable behavioral effects were also seen. The subjects sat slumped in the primate chair, emesis was frequently observed and upon returning to their home cage the subjects were typically unable to climb to their perch. In monkey M279 (+)-trans-Δ^9-THC had no effect on response rate at doses up to 30 mg/kg. No overt behavioral effects were observed from this drug in this subject at any dose. The potency ratio between the isomers in this subject was greater than 100 to 1. In monkey M287 the data for (+)-trans-Δ^9-THC were more variable. At 30 mg/kg a clear effect on response rates was seen. The effect was accompanied by observable difficulty in maintaining an upright seated posture in the primate chair and marked ataxia after returning to the home cage. These overt effects were similar to those seen with (-)-trans-Δ^9-THC. The effects of lower doses were within 2 s.d.'s of the average baseline response rate. (+)-trans-Δ^9-THC doses were administered to this subject in a descending order and during this testing baseline response rates were increasing. Thus, the effects of 3 and 10 mg/kg were even less different from control than are evident in the figure. We conclude that the minimal active dose of (+)-trans-Δ^9-THC in this subject was 30 mg/kg, resulting in a potency ratio between the isomers of about 100 to 1.

Overt behavior in dogs

The effects of the stereoisomers on the overt behavior of dogs is presented in Table 2. The (-)-isomers produced dose related changes in behavior as reported previously (12). The effects were usually maximal 30 min after i.v. administration. At 0.2 mg/kg both (-)-trans-Δ^9- and Δ^8-THC produced

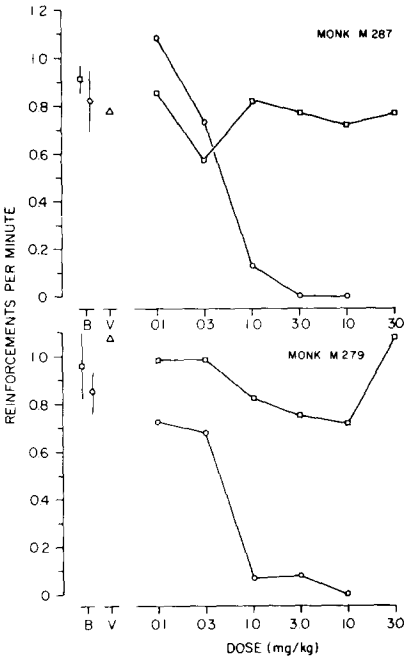

Figure 1

The effects of (+)- and (-)-trans-Δ^9-THC on rate of reinforcement in two squirrel monkeys responding a variable interval 60 sec schedule. The values at B represent the mean baseline reinforcement rate ± 1 s.d. during the testing of each drug. The values at V are the effects of vehicle injections. The circles represent the effects of (-)-trans-Δ^9-THC and the squares represent the effects of (+)-trans-Δ^9-THC.

the aggregate effects (tail-tucked, static ataxia, depressed activity, etc.) that normally typify cannabinoids. The (+) unnatural isomers differed from the (-)-isomers, both quantitatively and qualitatively. The (+)-isomers were considerably less active than the (-)-isomers. At doses less than 2.0 mg/kg the (+)-isomers were minimally effective and lacked dose-responsiveness. Absence of dose-related effects by the (+)-isomers was due, at least in part, to variability not normally seen with the static-ataxia test. For example, 0.2 mg/kg of (+)-trans-Δ^9-THC produced classical cannabinoid actions in one animal but was almost completely devoid of activity in the other two dogs.

The (+)-isomer also differed from the (-)-isomers in that the major effect seen was depression of spontaneous activity rather than the usual composite of behavioral signs. Some animals did exhibit the other signs (ataxia, tail tuck, etc.) but all were never present in animals tested with the (+)-isomers. The dogs that received the higher doses of (+)-cis-Δ^9-THC and (+)-trans-Δ^8-

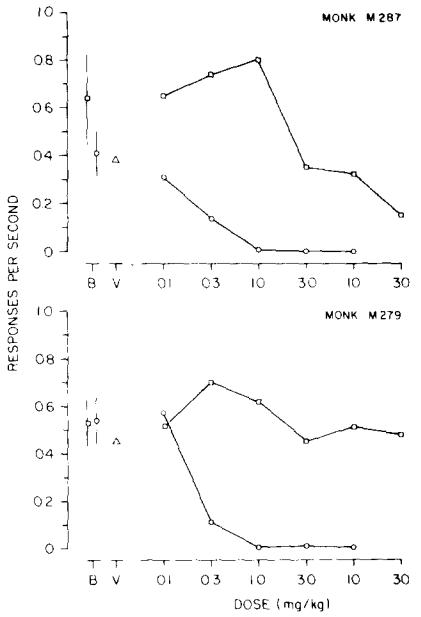

Figure 2

The effects of (+)- and (-)-trans-Δ^9-THC on response rates in two squirrel monkeys responding on a variable interval 60 sec. schedule. The values at B represent the mean baseline reinforcement rate ± 1 s.d. during the testing of each drug. The values at V are the effects of vehicle injections. The circles represent the effects of (-)-trans-Δ^9-THC and the squares represent the effects of (+)-trans-Δ^9-THC.

THC were prostrate within 5-10 min of the injection which made the observation for the other signs difficult. There appeared to be no difference in the (+)-cis- and (+)-trans isomers.

Since the limited supply of (+)-isomers restricted the number of high doses that could be tested, there were insufficient data for determination of exact dose-ratios. However, it is evident that the (+)-isomers are less active than the (-)-isomers, probably by ten fold.

Body temperature in mice

The (-)-trans isomers of Δ^8- and Δ^9-THC produced dose-related decreases in body temperature similar to those described by Haavik et al. (13). Hypothermia was observed at all time points and maximum effects were always found at either 40 or 60 min after the injection. The (+)-isomers also produced hypothermia that was temporally similar to the (-)-isomers. The maxi-

Table 2

Effects of the Stereoisomers on Overt Behavior in Dogs[a]

	Dose mg/kg							
Cannabinoid	0.2	0.4	0.5	1.0	1.25	2.0	4.0	5.0
(-)-trans-Δ^9-THC	3(5)	4[b]						
(+)-trans-Δ^9-THC	1(3)			1(2)		1(1)		6(1)[c]
(+)-cis-Δ^9-THC	1(2)		0(3)	1(2)	1(1)	0(3)		
(-)-trans-Δ^8-THC	2(2)	3(3)		5[b]				
(+)-trans-Δ^8-THC	1(1)		0(1)	1(3)		3(1)	6(1)[c]	

[a]Animals were scored as described in Table 1. Mean scores were presented with the number of animals in parenthesis.

[b]Data presented in reference 12.

[c]Prostrate, other cannabinoid effects were not seen.

mum hypothermia produced by the stereoisomers is presented in Table 3. The vehicle produced a slight decrease in temperature at 10 min but by 40 or 60 min this hypothermia was approximately 0.5°. Potency ratios were calculated according to an unsymmetrical-dose parallel-line assay because of unequal replicates and lack of log-dose spacing (14). The mean potency ratio of (+)-trans-Δ^9-THC to (-)-trans-Δ^9-THC was 9.1 with 95% confidence limits to lie between 5.9 and 15.4. The dose-response lines did not deviate from parallelism. The mean potency ratio of (+)-trans-Δ^8-THC to (-)-trans-Δ^8-THC was 30.4 with 95% confidence limits of 22.3 and 48.4.

Spontaneous activity in mice

The stereoisomers of trans-Δ^9-THC decreased spontaneous activity in a similar fashion. The maximum effect was observed during the time period of 0-10 min. after each isomer and spontaneous activity returned to control levels after 50-60 min. Since maximal effects were observed at 0-10 min., data during this time period were used to calculate potency ratios. Although the time courses were similar, the potency of the isomers differed as shown in Table 4. The ED50 (95% confidence limits) for (-)-trans-Δ^9-THC was 2.6 mg/kg (1.3-5.1) and 14.6 mg/kg (9.1-23.5) for (+)-trans-Δ^9-THC. The potency ratio of the ED50's was determined to be 5.6. The limited supplies of the other isomers did not permit their evaluation.

Discussion

The (-)-trans-isomers of THC were more effective than the unnatural isomers in all the pharmacological tests. The greatest degree of stereo-

Table 3

Maximum Hypothermia Produced by the Stereoisomers of Δ^8- and Δ^9-THC in Mice[a]

	Mean ± S.E. (N)			
Dose (mg/kg)	(-)-trans-Δ^9-THC	(+)-trans-Δ^9-THC	(-)-trans-Δ^8-THC	(+)-trans-Δ^8-THC
Vehicle	1.7 ± .1 (30)	1.7 ± .1 (3)	1.4 ± .5 (6)	.8 ± .2 (6)
2	----	----	2.8 ± .3 (6)	----
4	2.9 ± .4 (11)	----	4.4 ± .4 (6)	----
8	3.9 ± .4 (20)	----	5.7 ± .4 (6)	-----
16	4.9 ± .4 (31)	1.3 ± .6 (6)	----	----
40	----	2.8 ± .3 (12)	----	1.7 ± .5 (6)
60	----	3.7 ± .4 (12)	----	2.8 ± .3 (6)
80	----	4.3 ± .5 (16)	----	3.4 ± .3 (6)

[a]Maximum temperature change was determined for each mouse by substracting postinjection temperatures from the preinjection temperature.

Table 4

Effect of the Stereoisomers of Δ^9-THC on Spontaneous Activity in Mice[a]

	Percent of Vehicle	
Dose (mg/kg)	(-)-Δ^9-THC	(+)-Δ^9-THC
2	59 (6)	---
4	34 (8)	---
6	25 (5)	---
8	12 (7)	---
10	---	75 (6)
20	---	25 (7)
30	---	14 (5)
40	---	4 (8)

[a]Represents activity for 0-10 min. after i.v. administration. The vehicle treated animals (N=32 pairs of mice) had 103 ± 7 (mean ± SE) interruptions of the photocells. Results are expressed as % control with the number of pairs per group in parenthesis.

specificity was observed in the monkey where the potency ratio of (+)- to (-)-trans-Δ^9-THC was at least 100. Edery et al. (4) also found (-)-trans-Δ^9-THC to be considerably more potent than (+)-trans-Δ^9-THC in the rhesus monkey but no estimate of potency ratio could be made since the doses of the isomers tested never differed by more than 10-20 fold. There was less stereospecificity in the dog static-ataxia test than in the schedule-controlled behavior of monkeys. The unnatural isomers, both cis and trans, were less effective than the corresponding natural isomer at all doses tested. At very high doses, the unnatural isomers produced prostration that may have resulted from a generalized, nonspecific depression of the central nervous system. The unnatural isomers also produced the classical static-ataxia that is unique for cannabinoids. However, this effect was only seen in approximately one of three animals and occurred independent of dose. This variability was not seen with the natural isomers, nor has it been observed previously (10, 12, 15, 16) It is not possible to calculate potency ratios from these data but a conservative estimate would be a ten-fold difference.

The THC's also exhibited less stereospecificity in mice than in monkeys. In addition, the effects on hypothermia appeared to be more stereospecific than those on spontaneous activity. Jones et al. (5) had previously reported (-)-trans-Δ^9-THC to be 13 times more active than (+)-trans-Δ^9-THC in the mouse ring test. However, they were concerned about their potency estimate since their (+)-isomer contained approximately 3% of the (-)-isomer. We have shown that unnatural isomers which are probably 100% pure (stereospecific synthesis) are also active in the mouse and are approximately one-tenth or one-fifth as

active as the natural isomers. Also, these low potency ratios would not be altered even if the unnatural isomers contained 2-3% of the (-)-isomer.

It appears that the degree of pharmacological stereospecificity of the cannabinoids depends upon either species or pharmacological effect used or a combination of both. It may be that the actions of THC in the monkey, as measured by changes in schedule-controlled behavior, represent interactions only with a specific receptor, whereas the actions in the dogs and mice may represent a composite of receptor and nonreceptor mechanisms. It would be highly improbable that all the various actions of any drug would be mediated through receptors.

Potency ratios of stereoisomers differ with respect to species, test system and the individual laboratory for most drugs, not just for the cannabinoids. Jacquet et al. (17) have proposed two types of opiate receptors based upon their observations in different test systems. Nicotine's stereospecificity also spans a wide range (potency ratios of 2-100) depending upon the experimental conditions (18).

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