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Separation of the discriminative stimulus effects of stereoisomers of Δ^2- and Δ^3-tetrahydrocannabinols in pigeons [1]

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Pigeons, trained to discriminate between the presence or absence of Δ^1-tetrahydrocannabinol (THC) (I) (0.56 mg/kg), were tested with (1S,4R)-Δ^2-THC (II) (1-17.5 mg/kg), with the C-1 epimers of (4R)-Δ^2-THC acetate, namely (1S,4R)-Δ^2-THC acetate (IIIA) (3-17.5 mg/kg) and (1R,4R)-Δ^2-THC acetate (IIIB) (1-17.5 mg/kg) and with the enantiomers of Δ^3-THC acetate, namely (1S)-Δ^3-THC acetate (IVA) (1-10 mg/kg) and (1R)-Δ^3-THC acetate (IVB) (3-30 mg/kg). The results indicated that (I) was considerably more potent than any of the other compounds evaluated (ED_{50} of compound I = 0.18 and 0.25 mg/kg at the two post-injection intervals examined, 90 and 270 min, respectively). Furthermore, of the two Δ^2-THC acetates, compound (IIIB) was active whereas compound (IIIA) was not in comparable doses. The parent phenol of compound (IIIA), namely (II), was also inactive. Comparison of the pair of enantiomers, (IVA) and (IVB), showed the former to be significantly more potent than the latter. We have thus shown that the Δ^1-THC-like cue properties are separated in the stereoisomers of Δ^2- and Δ^3-THC.

Tetrahydrocannabinol (THC); THC-cue; Stereochemistry; Δ^2-THC; Δ^3-THC; (Pigeon)

1. Introduction

An important though still unresolved problem of cannabinoid structure activity relationships (SAR) is the extent of stereospecificity. Very few cannabinoid stereoisomers have been tested so far and the results are not unequivocal (for a recent review see Mechoulam et al., 1987). Most of the results presented so far deal with either enantiomers of Δ^1-THC * (Δ^9-THC) and Δ^6-THC (Δ^8-THC) or of their homologs. A recent observation, possibly of a general nature, is that there is a complete separation of activity in pure, crystalline enantiomers of the dimethyl heptyl homolog of 7-hydroxy-Δ^6-THC. The enantiomer with the 'natural' configuration (3R,4R) is a very potent cannabimimetic, while the (3S,4S) enantiomer is inactive in several animal tests, including drug discrimination, at doses thousands of times greater than the ED_{50} of the active enantiomer (Mechoulam et al., 1987). Our group has also published the result of several investigations on the C-1 epimers

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* THC = tetrahydrocannabinol.

of hexahydrocannabinol and 7-hydroxyhexahydrocannabinol (Mechoulam et al., 1980; Järbe et al., 1986), in which the compounds with an equatorial C-1 methyl group were found to be much more active than those with an axial methyl group.

In the present paper we present and analyze data on two yet uninvestigated types of THCs, namely the C-1 epimers of (4R)-Δ^2-THC acetate and the two enantiomers of Δ^3-THC acetate. These two types of THC stereoisomers were synthesized in 1984 (Srebnik et al., 1984). Δ^2-THCs have not been tested so far in either animals or man. Racemic Δ^3-THC has a long history of chemical and pharmacological investigation. It was first synthesized by Adams and Baker (1940) and Ghosh et al. (1940) and was shown to have cannabimimetic activity in animals and man (for reviews on the early work see Adams, 1942; Todd, 1946; Loewe, 1950). Until the natural THC, namely Δ^1-THC (Δ^9-THC), was identified in 1964 (Gaoni and Mechoulam), racemic Δ^3-THC was widely used as the standard active cannabinoid/cannabimimetic.

The enantiomers of Δ^3-THC have not yet been studied in animals. However, a comparative study in humans has been reported (Hollister et al., 1987). The (1S) enantiomer has definite psychic actions, qualitatively similar to those of Δ^1-THC, but quantitatively less potent (1 : 3 to 1 : 6). The (1R) enantiomer was not active. However, it could not be tested in high doses due to limited availability of drugs.

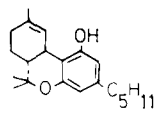
2. Materials and methods

2.1. Drug discrimination training

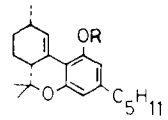
Eight male White Carneaux pigeons (Estuna AB, Sweden and Palmetto, Sumter, U.S.A.) were trained to discriminate between (−)-Δ^1-THC and vehicle (5% propylene glycol, 2% Tween-80 and 93% physiologic saline (v/v)), according to published procedures (Hiltunen and Järbe, 1986; Järbe and Hiltunen, 1987). The training dose was 0.56 mg/kg (1 ml/kg) administered intramuscularly (i.m.) 90 min prior to the onset of the training session. The experimental chambers, adapted after Ferster and Skinner (1957), containing two response keys separated by a recess in which grain rewards (4 s access to chicken pellets) could be presented. The weights of the animals were reduced to about 80% of their free feeding weights. Which key was correct on a given training session depended upon whether Δ^1-THC or vehicle had been administered prior to the session. The schedule of reinforcement used was fixed ratio 15 (FR-15), i.e. the reward was available during 4 s when 15 key-pecking responses had been registered on the key appropriate for the administration (drug or no drug). Responses on the inappropriate key were also recorded but had no programmed consequence. The session ended when the pigeons had received 52 rewards or 20 min had elapsed, whichever occurred first. The animals were trained 3 days a week.

2.2. Drug discrimination testing

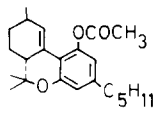
Once trained the animals were tested once a week (Fridays), provided that the correct base-line response was maintained, i.e. no more than 29



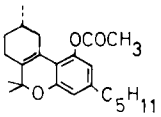
Δ^1-THC (I)

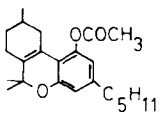


(1S,4R)-Δ^2-THC, R = H (II)
(1S,4R)-Δ^2-THC acetate, R = $COCH_3$ (IIIA)



(1R,4R)-Δ^2-THC acetate (IIIB)





(1S)-Δ^3-THC acetate (IVA) (1R)-Δ^3-THC acetate (IVB)

Fig. 1. Chemical structures of the tetrahydrocannabinols (THC) examined in this study.

pecking responses should be made before the first reward in the regular training sessions occurring on Mondays and Wednesdays. In the drug discrimination test, the session ended after six rewards had been obtained or 20 min had elapsed since the initiation of the test. Both keys were operable throughout the test (six trials) and consequently pecking (FR-15) on either of the two keys produced the 4 s access to the hopper filled with chicken pellets. Two intervals viz. 90 and 270 min after a single administration were examined after each drug dose. The birds were kept in their home boxes between the two test intervals. The test drugs were given i.m. and were studied in an unsystematic mixed order. Suspensions were prepared shortly before their administration and volumes varied depending upon the amounts to be injected (see below).

2.3. Drugs

The Δ^2- and Δ^3-cannabinoids were prepared according to published procedures (Srebnik et al., 1984). According to the dibenzopyran nomenclature, Δ^2- and Δ^3-THC equal $\Delta^{10,10a}$-THC and $\Delta^{10a,6a}$-THC, respectively. Suspensions were prepared as described previously (Järbe et al., 1981). The chemical structures of the compounds used in this study are given in fig. 1.

TABLE 1

Results of the substitution test with Δ^2- and Δ^3-THC compounds in pigeons trained to discriminate between 0.56 mg/kg of (−)-Δ^1-THC and vehicle. The training sessions occurred 90 min after drug administration. In the test sessions the birds were tested at two times for each dose, i.e. 90 min and 270 min after a single i.m. injection. Agents: (I) = Δ^1-THC; (II) = (1S,4R)-Δ^2-THC; (IIIA) = (1S,4R)-Δ^2-THC acetate; (IIIB) = (1R,4R)-Δ^2-THC acetate; (IVA) = (1S)-Δ^3-THC acetate; and (IVB) = (1R)-Δ^3-THC acetate. n = number of pigeons examined per dose. %RDP = percentage of pecking responses that the birds made on the key associated with drug training; [a] one animal in each of these tests did not complete at least 15 pecking responses on either of the keys and hence did not obtain any reward (and hence was not considered in the calculations of %RDP). Response time = the quotient between the time needed to obtain the first six rewards in vehicle sessions and the time taken in test sessions; scores below or above 1 indicate a longer or shorter time, respectively, taken to receive the rewards (±S.E.M.). All the birds examined at a particular dose were considered in the analysis. The eight birds took 88.2 (±3.2) s (mean±S.E.M.) to obtain the six rewards in the regular non-drug training sessions. For some of the higher test doses, the volumes given i.m. were 2-3 ml/kg and the amount of Tween-80 used was increased to 3-4% at the expense of saline. [b] $P \leq 0.05$; [c] $P \leq 0.01$ (Student's t-statistics for paired comparisons, two-tailed, df = n − 1).

Agent	Dose (mg/kg)	n	%RDP		Response time	
			(90 min)	(270 min)	(90 min)	(270 min)
(I)	0.56	8	100	81.3	0.84 (0.12)	1.12 (0.06)
(II)	1	6	0	0	1.08 (0.03)	1.15 (0.04)
	3	6	0	0	0.95 (0.09)	1.11 (0.06)
	10	4	0	0	0.92 (0.06)	1.11 (0.05)
	17.5	2	0 [c]	0	0.87 (0.16)	0.84 (0.22)
(IIIA)	3	5	0	0	1.09 (0.12)	1.10 (0.11)
	10	5	19.6	2.4	0.68 (0.17)	0.97 (0.10)
	17.5	4	0 [c]	0	0.57 (0.20)	1.14 (0.07)
(IIIB)	1	3	0	0	0.96 (0.01)	0.82 (0.13)
	3	4	0.3	0	0.62 (0.18)	0.86 (0.14)
	10	3	100 [a]	100	0.31 (0.20)	0.93 (0.15)
	17.5	4	100 [a]	100	0.42 (0.25)	0.64 (0.15) [b]
(IVA)	1	4	0	0	1.00 (0.04)	1.07 (0.06)
	3	4	41.7	4.2	0.93 (0.08)	1.01 (0.04)
	5.6	3	18.6	41.7	0.89 (0.15)	0.94 (0.15)
	10	4	75	100	0.50 (0.20)	0.78 (0.22)
(IVB)	3	4	0	4.2	0.70 (0.05)	0.97 (0.05)
	10	4	42.1	0.6	0.85 (0.07)	0.94 (0.06)
	17.5	4	54.2	50	0.60 (0.17)	0.89 (0.06)
	30	4	94.4 [a]	97.3 [a]	0.58 (0.26)	0.66 (0.26)

3. Results

Table 1 summarizes the results of the generalization tests obtained with eight pigeons trained to discriminate between 0.56 mg/kg of (−)-Δ^1-THC (I) and vehicle. It shows that, in a test with the training dose of Δ^1-THC (I), all pecking responses were made at the key associated with drug training at the 90 min post-injection (p.i.) test interval, and that more than 80% of the responses occurred at the key associated with drug training (%RDP) at the later interval of 270 min p.i. The rate of responding, i.e. the time taken to complete a cycle of the first six rewards (right hand section of table 1) is expressed as the quotient between the time used to obtain the initial six rewards in non-drug/vehicle training sessions divided by the time needed in the drug tests. A comparison between the response times recorded after Δ^1-THC administration and after the highest dose of the other compounds revealed only one statistically significant difference viz. that of compound (IIIB) at the 270 min test interval (cf. table 1).

Furthermore, in sufficient doses (1R,4R)-Δ^2-THC acetate (IIIB) also elicited drug-appropriate responses (i.e. pecking at the key associated with drug training) whereas comparable doses of (1S,4R)-Δ^2-THC acetate (IIIA) did not to an appreciable degree. The difference in %RDP between Δ^1-THC and compound (IIIA) was statistically significant. In tests with (1S,4R)-Δ^2-THC (II) (dose range 1 to 17.5 mg/kg) only non-drug appropriate responses occurred, which were also statistically different from the %RDP test results with Δ^1-THC.

This table also shows that the drug-(THC)-response was generalized to both enantiomers of Δ^3-THC acetate, the (1S)-compound (IVA) being more potent than the (1R)-Δ^3-THC acetate (IVB) (cf. tables 1 and 2).

The potency relationship between the compounds, as given by the median dose effect (ED_{50}) estimates, is shown in table 2. The ED_{50} values were derived from logarithmic regression analyses and the fit (r) of the data to the regression line is given within the parentheses. Apparently Δ^1-THC (I) was considerably more potent than any of the other compounds investigated. Further, compound (IVB) was significantly less active than compounds (IIIB) and (IVA) at both test intervals; the latter two compounds did not differ significantly from each other.

During the training sessions the pigeons averaged (± S.E.M.) 95.9 (1.8) and 97.8 (1.7) % correct initial selections after Δ^1-THC and vehicle administration, respectively (n = 8). Data concerning the initial six rewards have been presented elsewhere (Hiltunen and Järbe, 1986; Järbe et al., 1986; Järbe and Hiltunen, 1987).

TABLE 2

The ED_{50} (mg/kg) values and, within parentheses, the corresponding fits (r) for the logarithmic regressions on which the median dose effect estimates were based. The data for compound (I) are the means of the determinations described in Hiltunen and Järbe (1986), Järbe and Hiltunen (1987), and Järbe et al. (1986) as well as one published experiment by Järbe and Hiltunen. The basic data in all of these calculations are the average percentages of responses made to the drug-associated key (%RDP) for each particular dose and test interval examined. [a] $P \leq 0.05$; [b] $P \leq 0.02$; [c] $P \leq 0.01$ (Student's t-statistics for paired contrasts, two-tailed, df = n − 1) as compared to the %RDP of compound (IVB); compound (I) was not included in these analysis. Comparisons between compounds (IIIB) and (IVA) were non-significant ($P > 0.05$) concerning both injection-test intervals.

Compound	90 min	270 min
(I)	0.18 (0.95)	0.25 (0.96)
(IIIB)	4.78 (0.92) [b]	4.79 (0.92) [c]
(IVA)	6.63 (0.81) [a]	5.00 (0.88) [c]
(IVB)	11.90 (0.98)	15.23 (0.84)

4. Discussion

The observation that the stereoisomers tested differed in their potency to elicit THC-appropriate responses indicates that the stereoisomers of Δ^2- and Δ^3-THC exert different effects. However, it is only when the test compound mimics the 'main' effects of the training drug that there will be a response generalization between the two drug stimuli, as established in drug (D) vs. non-drug (N) training, in this case 0.56 mg/kg Δ^1-THC vs. vehicle. Hence lack of generalization simply means the absence of characteristic effects during the D vs. N training (Järbe and Swedberg, 1982), but

does not carry any further implication about the discriminatory effects of the test compound.

A separation of activity between the C-1 epimers of (4R)-Δ^2-THC (compounds IIIA and IIIB) was observed. The lack of cannabimimetic, Δ^1-THC-like cue activity in the (1S) pseudoaxial epimer (IIIA) is in accord with previous observations (Mechoulam et al., 1980; Järbe et al., 1986) made with hexahydrocannabinol or 7-hydroxy-hexahydrocannabinol, in which the axial epimers also exhibited no activity or lower activity when compared to the equatorial epimers. It seems therefore that the requirement that the C-1 substituent be in the plane of the cyclohexane ring of the cannabinoid is a general one.

The two enantiomers of Δ^3-THC acetate were observed to differ only in potency since generalization occurred with sufficient doses of both compounds. Racemic Δ^3-THC has previously been observed to induce effects similar to but less intense than those induced by Δ^1-THC both in animals and man (Hollister, 1974; Mechoulam and Edery, 1973; Sofia, 1978). As indicated above, (1S)-Δ^3-THC (IVA) has recently been found to be active in man at doses 3-6 times greater than those of Δ^1-THC needed to produce the same effects; (1R)-Δ^3-THC (IVB) was found to be inactive (Hollister et al., 1987). This is in contrast with the present data, though it seems possible that, in the human experiments, the cannabimimetic effects of (1R)-Δ^3-THC (IVB) could have been observed at doses higher than those that were used.

The ratio of activity between Δ^1-THC and (1S)-Δ^3-THC is greater in pigeons than in humans (in whom, however, only the parent phenol rather than the acetate was tested). Whatever the reasons for this species difference, it is obvious that the change of the position of the double bond produces a loss or at the least a change of activity in both species.

Δ^2-THC has not been tested before. Hence it remains to be determined whether the difference in potency between the active isomer (IIIB) and Δ^1-THC (the ED_{50} values varied by an order of 19 [270 min p.i.] or 27 [90 min p.i.]) will also be observed in other species. The lack of substitution with compound (IIIA) indicates a separation of the cannabimimetic activity in the Δ^2-THC epimers. The parent phenol of (IIIA), i.e. compound (II), also failed to show generalization, indicating a lack of cannabimimetic, Δ^1-THC-like cue activity. Whether doses of compounds (IIIA) and (II) that are higher than those that could be tested here would result in response generalization remains to be determined.

It would be of interest to examine cannabinoids in which the double bond is located at positions 4 and 5 to determine whether the Δ^1 position is indeed the location which results in the most marked cannabimimetic effects. Location of the double bond at position 6 (Δ^8-THC following dibenzopyran nomenclature) produces effects similar to those of Δ^1-THC but the potency of Δ^6-THC is lower than that of Δ^1-THC (e.g. Järbe et al., 1977; Mechoulam and Edery, 1973).

Acknowledgements

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