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THE ORAL ACTIVITY OF Δ'-TETRAHYDROCANNABINOL AND ITS DEPENDENCE ON PROSTAGLANDIN E_2

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1 Δ'-*trans*-Tetrahydrocannabinol (THC) is more active orally in mice than previously thought, as cataleptic responses occur at doses from 0.06 mg/kg upwards, with peak activity at 2 to 4 h after dosing. These doses and peaks correspond well with the effects in man.

2 Comparison with chlorpromazine in mice shows that chlorpromazine and THC are equipotent as cataleptics during the first 2 h after dosing; thereafter the THC activity increases to a peak when it is 5.67 times as active as chlorpromazine.

3 The cataleptic effect of THC is abolished by aspirin, indomethacin, diflunisal and phenylbutazone which inhibit the biosynthesis of prostaglandins and is restored by exogenous prostaglandin E_2 (PGE_2) but not PGE_1 and $PGF_{2\alpha}$. This suggests that the effect of THC depends upon the presence of PGE_2.

4 In contrast, the cataleptic effect of chlorpromazine is not affected by pretreatment with aspirin.

5 THC is very much less active intraperitoneally than orally; our results suggest this is not due to poor absorption or extraction into fat depots.

6 Cannabidiol has no cataleptic effect.

Introduction

Cannabis users may take the drug in one of four ways: (a) smoking of reefers, (b) smoking through a water pipe (Kephalas, Kiburis, Michael, Miras & Papadakis, 1976), (c) ingestion as a milky drink, (d) eaten as a buttery confection (Graham & Li, 1976), that is, the active material is absorbed either from the lungs or the gastrointestinal tract. Surprisingly enough, most experiments on the pharmacological, neurochemical and behavioural effects of cannabinoids in animals have been by intraperitoneal (i.p.) injection (Ho, Fritchie, Englert, McIsaac & Idanpaan-Heikilla, 1971). There are two serious disadvantages to this route. Firstly, although (−)Δ'-*trans*-tetrahydrocannabinol (THC) is active intraperitoneally, the doses required are far in excess of the corresponding effective oral dose in man (Isbell, Gorodetsky, Jasinski, Clausen, von Spulak & Korte, 1967; Hollister, Richards & Gillespie, 1968; Jones, 1971; Kiplinger & Manno, 1971; Perez-Reyes, Lipton, Timmons, Wall, Brine & Davis, 1973; Davies, Weatherstone, Graham & Griffiths, 1974; Noyes, Brunk, Baram & Canter, 1976; Graham, Davies, Seaton & Weatherstone, 1976). Secondly, intraperitoneal injections of THC induce the abdominal constriction response which is generally accepted as an indication of pain (Collier, Dinneen, Johnson & Schneider, 1968). Such an effect could modify the neurochemical and behavioural responses to THC.

Our first objective was therefore to compare the cataleptic activity of THC given intraperitoneally with that given orally; cannabidiol (CBD) was also compared in the same way. In addition we decided to determine cataleptic activity of THC in food-deprived and well-fed mice and in small and obese ones, since it has been claimed that the low intraperitoneal activity is due to lack of absorption (Ho *et al.*, 1971) and to accumulation in peritoneal fat depots (Malor, Chester & Jackson, 1976). We also determined the LD_{50} of THC by both oral and intraperitoneal routes, and for comparison purposes the cataleptic activity of chlorpromazine orally.

As already stated, cannabis is normally ingested from the lungs or the gastrointestinal tract and as the lungs (Piper & Vane, 1969) and the stomach (Bennett, Murray & Wyllie, 1968) are easily provoked to release prostaglandins, we decided to investigate the effect of some inhibitors of cyclo-oxygenase (the enzyme that catalyses the conversion of arachidonic acid to cyclic endoperoxide) on the response to THC and their modification by injected prostaglandins E_1 (PGE_1), E_2 (PGE_2) or $F_{2\alpha}$ ($PGF_{2\alpha}$).

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Methods

Cataleptic activity

Groups of 20 male LACA Tuck No. 1 strain albino mice weighing 18 to 22 g, unless otherwise stated, were maintained on a 12 h light–dark cycle. Food (oxoid diet 44B) was withdrawn 18 h before and during the test, but water was freely available. The mice were kept in a cabinet maintained at 30 to 32°C, to avoid the hypothermic effect of THC (Pertwee, 1972) and laboratory noise was kept to a minimum. THC and CBD were suspended in Tween 80 (2.5% in water) and injected (0.20 ml/20 g body weight of mouse) directly into the stomach with a hypodermic syringe fitted with a blunt serum needle.

The test was performed as described by Pertwee (1972). The dosed mice were transferred to the wire ring, at selected times after dosing, and left on the ring for 5 min. The sum of the times during which each mouse remained motionless in this 5 min period was recorded, and the group mean calculated together with the standard error of the mean (s.e. mean). Catalepsy was expressed as that percentage of the total time spent on the ring during which the animal remained motionless. Controls and mice pretreated with inactive compounds did not stay on the ring for more than 7.2 ± 2.0 s ($n = 40$). Differences between drug and control groups were evaluated by Student's t test.

Pretreated mice were tested at 10 and 30 min and at 1, 2, 4 and 6 h after dosing, taking care however not to re-test any individual mouse without a minimum interval of 2 h, otherwise it would give too high a cataleptic response (Pertwee, 1972). We have expressed potency as the cataleptic dose fifty (CD_{50}) which is the dose in mg/kg required for 50% catalepsy and calculated from the graph relating probit percentage catalepsy (ordinate scale) against log dose (abscissa scale). The fiducial limits of the CD_{50}s were calculated by the method of Litchfield & Wilcoxon (1949).

Some experiments were carried out with mice weighing over 33 g to see if the heavier mice were less sensitive.

Toxicity

Deaths occurring within 8 days of single doses of THC after intraperitoneal and oral administration were recorded and the toxicity expressed as the LD_{50}, the dose in mg/kg causing death in 50% of the mice within 8 days of a single dose.

Prostaglandin inhibition

Aspirin in doses from 10 to 100 mg/kg was given orally in mucilage of tragacanth to female mice; 3 min later THC (25 mg/kg, orally) was given to the mice and the cataleptic effect measured 120 min later. Other non-steroidal anti-inflammatory compounds were also tested in a similar manner; phenylbutazone and indomethacin in tragacanth mucilage and diflunisal in 0.5% methyl cellulose-20, B.P.C. 1973 (Stone, van Arman, Lotti, Minsker, Risley, Bagdon, Bokelman, Jensen, Mendlowski, Tate, Peck, Zwickey & McKinney, 1977). This series of tests was repeated to include administration intraperitoneally of the prostaglandins 100 min after giving the THC; the cataleptic effect was measured at 120 min as before. The prostaglandins PGE_1, PGE_2 and $PGF_{2\alpha}$ (3.3 μg/kg) were each used in separate series of tests and the results analysed statistically. We also tested the effect of chlorpromazine in aspirin-treated mice.

Drugs

All dilutions were made with distilled water. THC was prepared as a stock solution containing 10 mg/ml in 2.5% w/v Tween 80. All other solutions or suspensions were made up immediately before use. The following drugs were used: THC and CBD (Makor Chemical Co., Ltd., Jerusalem), aspirin (BDH Ltd.), indomethacin and phenylbutazone (Sigma Chemical Co.), diflunisal (Development Laboratories, Merck, Sharp & Dohme), prostaglandins (Cambrian Chemicals Ltd.) and chlorpromazine (May & Baker Ltd.).

Results

Cataleptic activities

Δ'-trans-*Tetrahydrocannabinol* (THC) was given in doses from 20 mg to 0.06 mg/kg orally and from 100 mg to 0.1 mg/kg intraperitoneally. From these results recorded, with the exception of the 10 min readings, in Table 1 it will be seen that with oral doses of 5 to 20 mg/kg the onset of the effect was rapid (within 10 min) and the effect lasted for 6 h. With lower doses of 0.06 to 2.5 mg, the peak effect took much longer to develop but significant effects were found. The intraperitoneal route required much higher doses of 10 to 25 mg/kg THC.

Cannabidiol (CBD) was inactive orally at all the doses tested (Table 1) up to 100 mg/kg.

Chlorpromazine was active orally at 4, 2 and 1 mg/kg (Table 1).

Intraperitoneal THC was found to be equi-active in food-deprived and well-fed mice and in obese and small mice.

Acute toxicities

The acute toxicity tests with THC intraperitoneally and orally showed that 8 day LD_{50}s are approxi-

mately equal by both routes (Table 2) namely, 620 mg/kg orally and 540 mg/kg intraperitoneally.

Prostaglandin synthesis inhibitors

From the results given in Table 3 it will be seen that with doses of 10 to 100 mg/kg aspirin inhibited the response to THC (25 mg/kg) in a dose-dependent manner. THC 25 mg/kg was given to these female mice to equate approximately with 10 mg/kg in male mice (Table 1). Indomethacin, phenylbutazone and diflunisal also inhibited the response to THC, but chlorpromazine (8 mg/kg orally) had the same cataleptic effect in both normal (87.5 ± 3.37% catalepsy; $n = 4$) and in aspirin (100 mg/kg orally) pretreated mice (90.3 ± 2.52% catalepsy; $n = 4$). Prostaglandin E_2 (3.3 μg/kg i.p.) but not E_1 or $F_{2\alpha}$ abolished the reversal of the cataleptic response to THC by aspirin, indomethacin, phenylbutazone and diflunisal (Table 4). We found that very high doses of aspirin (600 mg/kg orally) and of diflunisal (100 mg/kg orally) did not prevent the cataleptic effect of THC; we cannot explain this reversal of action.

Discussion

The most interesting result of our work is the possibility that the cataleptic response in mice to oral doses of THC depends on the ability of the gastrointestinal

Table 1 Percentage catalepsy at different times after oral and intraperitoneal administration of Δ'-*trans*-tetrahydrocannabinol (THC), cannabidiol (CBD) and chlorpromazine (Cpz) to groups of male albino mice (Tuck No. 1 strain) at 30–32°C in the ring test

			Mean % catalepsy (±s.e. mean)				
Compound	*Dose (mg/kg)*	*Route*	*30 min*	*1 h*	*2 h*	*4 h*	*6 h*
THC	20	oral	81 ± 8 (11)*	83 ± 3 (11)*	81 ± 2 (11)*	76 ± 3 (3)*	60 ± 7 (12)*
	10		84 ± 6 (8)*	84 ± 7 (7)*	88 ± 6 (15)*	67 ± 7 (8)*	55 ± 9 (9)*
	5		69 ± 14 (5)*	65 ± 7 (8)*	84 ± 3 (11)*	71 ± 8 (9)*	47 ± 8 (9)*
	2.5		28 ± 5 (7)*	40 ± 6 (6)*	79 ± 4 (5)*	79 ± 4 (5)*	65 ± 6 (5)*
	1.25		12 ± 6 (5) NS	15 ± 7 (7) NS	52 ± 7 (5)*	79 ± 4 (7)*	58 ± 8 (7)*
	0.625		11 ± 6 (5) NS	NT	48 ± 6 (5)*	75 ± 7 (3)*	20 ± 10 (7)*
	0.0625		NT	NT	10 ± 6 (7) NS	19 ± 10 (7)	NT
	100	i.p.	75 ± 6 (10)*	85 ± 5 (5)*	82 ± 7 (10)*	69 ± 10 (10)*	81 ± 4 (5)*
	50		66 ± 6 (13)*	75 ± 7 (7)*	49 ± 9 (10)*	39 ± 8 (14)*	60 ± 9 (10)*
	25		57 ± 8 (9)*	68 ± 10 (3)*	39 ± 9 (7)*	56 ± 7 (9)*	NT
	10		32 ± 13 (9)*	34 ± 14 (9)*	NT	15 ± 7 (9)	16 ± 8 (9)
	1.0		12 ± 10 (5)	3 ± 3 (5)	1 ± 1 (5)	0 (5)†	0 (5)†
	0.1		0 (10)†	0 (10)†	0 (10)†	0 (10)†	0 (10)†
CBD	100	oral	1 ± 1 (5)	5 ± 4 (5)	2 ± 2 (4)	4 ± 5 (5)	NT
	50		3 ± 1 (5)	16 ± 11 (7)	6 ± 3 (4)	1 ± 1 (5)	NT
	25		NT	NT	2 ± 1 (6)	1 ± 1 (6)	NT
	12.5		0 (10)†	0 (5)†	0 (10)†	0 (10)†	0 (5)†
	6.25		0 (10)†	0 (5)†	0 (10)†	0 (10)†	0 (5)†
	3.13		0 (10)†	0 (5)†	0 (10)†	0 (10)†	0 (5)†
Cpz	4	oral	54 ± 10 (6)*	72 ± 4 (12)*	82 ± 3 (11)*	79 ± 7 (6)*	76 ± 7 (9)*
	2		31 ± 7 (12)*	50 ± 9 (6)*	65 ± 7 (6)*	58 ± 8 (6)*	53 ± 3 (6)*
	1		14 ± 1 (7)*	24 ± 5 (11)*	46 ± 6 (5)*	36 ± 6 (8)*	31 ± 14 (5)*
Tween	25	oral	2 ± 1 (7)	3 ± 2 (7)	2 ± 2 (7)	1 ± 1 (7)	3 ± 2 (10)
	80	i.p.	37 ± 7 (6)*	35 ± 5 (6)*	35 ± 5 (6)*	27 ± 2 (6)	13 ± 3 (6)
Water	10 ml	oral	2 ± 1 (7)	2 ± 1 (7)	1 ± 1 (7)	3 ± 2 (40)	2 ± 1 (10)

Differences at each time after i.p. or oral administration between compound and the appropriate vehicle (Tween 80 for THC and CBD and water for Cpz) were evaluated by Student's t test. A significant difference ($P < 0.001$) is denoted by *. The number of mice is given in parentheses. † denotes that the mice jumped off the ring more than 5 times. NT = not tested. NS = $P > 0.05$. Tween 80 i.p. was compared with undosed controls, not shown as they did not differ from water orally.

tract to synthesize prostaglandin E_2. This is indicated, firstly, by the fact that pretreatment with the three prostaglandin synthetase inhibitors, aspirin, indomethacin and phenylbutazone, prevents the cataleptic response to THC, and secondly, by the fact that injection of very small doses of PGE_2 (equivalent to 3.3 ng per g body wt.) to mice restores their cataleptic response. In contrast PGE_1 and $PGF_{2\alpha}$ have no such restorative effect. In this connection it is interesting that Bennet *et al.* (1968) showed that mucosa of the human stomach contains 1 μg of PGE_2 per g wet wt. but found no evidence for the presence of PGE_1

Table 2 Acute 8-day mortalities after single doses of Δ'-*trans*-tetrahydrocannabinol (THC) and cannabidiol (CBD) by intraperitoneal or oral administration on day 1 to albino female mice weighing 18 to 24 g

Compound	*Route*	*Dose (mg/kg)*	*No. dosed*	*No. dying on Day 1*	*Day 3*	*Cumulative % mortality*	LD_{50} *(mg/kg)*
THC	i.p.	2000	3	3		100	
		500	3	1	2	66	ca. 540
	oral	2000	3	1	3	100	
		1000	5	0	5	100	
		500	7	0	1	14	ca. 620
CBD	i.p.	1000	5	2	5	100	
		500	5	0	4	80	
		250	10	0	1	10	ca. 380
	oral	2000	3	0	0	0	
		500	5	0	0	0	>2000
Tween 80	i.p.	4000	8	0	1	19	
	oral	400	8	0	0	0	
Untreated controls			7	0	0	0	

N.B. There were no deaths on days 2, 4, 5, 6, 7, or 8.

Table 3 The effect of some cyclo-oxygenase inhibitors on the cataleptic response in Tuck albino female mice to Δ'-*trans*-tetrahydrocannabinol (THC) orally

Compound[b]	*Dose (mg/kg)*	*Cataleptic score(s)*	*% reversal of catalepsy*	P[c]
Control[a]	—	249 ± 7 (22)	—	—
Aspirin	10	217 ± 7 (5)	13 ± 2	$0.01 < P < 0.05$
	25	158 ± 28 (5)	37 ± 9	<0.001
	50	138 ± 40 (5)	44 ± 13	<0.001
	100	62 ± 42 (23)	75 ± 14	<0.001
Indomethacin	15	103 ± 23 (9)	59 ± 8	<0.001
Phenylbutazone	100	89 ± 20 (4)	64 ± 7	<0.001
Diflunisal	2.5	222 ± 14 (5)	11 ± 5	$0.01 < P < 0.05$
	5	169 ± 16 (5)	32 ± 6	0.001
	10	109 ± 26 (7)	56 ± 10	0.001

Values are mean ± s.e. mean; number of mice given in parentheses.
All mice received THC 25 mg/kg orally at zero time.
[a] Controls received only THC 25 mg/kg orally; [b] Additional compounds were given 3 min later.
[c] Significance of differences between cataleptic scores for mice receiving THC only and those receiving THC and test compounds were evaluated by Student's *t* test.

or $PGF_{2\alpha}$. Further support for our conclusion is found from our experiments with the new non-steroidal anti-inflammatory compound diflunisal, which is similar to aspirin chemically and pharmacologically (Hannah, Ruyle, Jones, Matzuk, Kelly, Witzel, Holtz, Houser, Shen & Sarett, 1977) but which inhibits prostaglandin synthesis by reversible binding to cyclo-oxygenase (Majerus & Stanford, 1977), whereas aspirin inhibits irreversibly by covalent acetylation of the cyclo-oxygenase (Roth, Stanford & Majerus, 1975). As an anti-inflammatory and analgesic compound, diflunisal is 7.5 to 13 times as active as aspirin in laboratory animals (Stone *et al.*, 1977). However, in our experiments it is only about twice

Table 4 The effects of prostaglandin E_1 (PGE_1), PGE_2 and $PGF_{2\alpha}$ on the reversal of the cataleptic effect of oral Δ'-*trans*-tetrahydrocannabinol (THC) 25 mg/kg orally by some cyclo-oxygenase inhibitors in Tuck albino female mice

Treatment[a]	*% catalepsy s.e. mean (n)*	P[b]
1 THC alone	79.7 ± 4 (9)	
2 THC + aspirin 100 mg/kg	20.8 ± 14 (23)	<0.001
3 THC + aspirin 100 mg/kg + PGE_2	74.1 ± 4 (12)	>0.05
4 THC + aspirin 100 mg/kg + PGE_1	26.4 ± 9 (8)	<0.001
5 THC + aspirin 100 mg/kg + $PGF_{2\alpha}$	36.1 ± 9 (4)	<0.001
6 THC + diflunisal 100 mg/kg	36.3 ± 9 (7)	<0.001
7 THC + diflunisal 10 mg/kg + PGE_2	95.0 ± 3 (5)	>0.05
8 THC + indomethacin 15 mg/kg	34.3 ± 8 (9)	<0.001
9 THC + indomethacin 15 mg/kg + PGE_2	91.2 ± 4 (10)	>0.05
10 THC + phenylbutazone 100 mg/kg	30.0 ± 7 (4)	<0.001
11 THC + phenylbutazone 100 mg/kg + PGE_2	78 ± 4 (5)	>0.05
12 PGE_2 3.3 μg/kg alone	None (10)	

n = Number of mice.
[a] THC was given orally at zero time. Aspirin, phenylbutazone, indomethacin and diflunisal were given orally 3 min later. Prostaglandins 3.3 μg/kg were given i.p. at 100 min. The catalepsy was measured at 120 min.
[b] Significance of differences between % catalepsy for THC alone and THC with test compounds were evaluated by Student's *t* test.

Table 5 Cataleptic potency expressed as CD_{50} (the dose required to give 50% catalepsy) at different times after intraperitoneal or oral administration of Δ'-*trans*-tetrahydrocannabinol (THC) and chlorpromazine (Cpz)

Compound	*Route*	*Time (min)*	*CD_{50} (95% conf. limits)*	*Potency ratio cf. THC i.p.*	*Potency ratio cf. Cpz oral*
THC	i.p.	30	22.5 (16.6–30.4)	1.00	
		60	19.0 (15.2–23.7)		
		120	36.5 (29.2–45.6)		
		240	54.0 (41.5–70.2)		
		360	36.5 (29.1–46.2)		
THC	oral	30	3.90 (3.12–4.87)	5.77 (3.72–8.94)	0.92 (0.76–1.10)
		60	3.45 (2.92–4.07)	5.51 (4.41–6.88)	0.62 (0.47–0.80)
		120	0.80 (0.55–1.16)	45.6 (29.4–70.7)	1.49 (1.10–2.01)
		240	0.27 (0.18–0.40)	200 (125–320)	5.67 (4.25–7.14)
		360	ca. 6.6	ca. 5.5	ca. 3.0
Cpz	oral	30	3.60 (3.10–4.18)		1.00
		60	2.15 (1.79–2.58)		
		120	1.20 (0.96–1.50)		
		240	1.53 (1.47–1.59)		
		360	1.80 (1.44–2.25)		

CD_{50}s have been calculated from the data Table 1 by the method of Litchfield & Wilcoxon (1949); 95% confidence limits are shown in parentheses.

as effective as aspirin in reducing THC-induced catalepsy.

Our results with chlorpromazine are interesting as they show that the cataleptic effect does not depend on the availability of prostaglandins, and this suggests that it operates through a different mechanism from that of THC.

A comparison of the *oral* and *intraperitoneal* potencies expressed as CD_{50} (Table 5) of THC shows that the oral route always has the higher activity and the difference increases with time, so that 4 h after dosing, oral THC is 200 times as active as intraperitoneal THC (Table 5), which we think is due to the involvement of prostaglandin E_2 within the gastro-intestinal tract. The alternative explanation that cannabinoids are poorly absorbed from the peritoneum (Ho, *et al.*, 1971) is contradicted by our results on acute toxicity (Table 2). Although the number of animals involved is small, our results agree well with the values of 454 mg/kg and 481 mg/kg (i.p.) cited by Graham (1976). Neither do we think that intraperitoneal THC is taken up by the abdominal fat stores as suggested by Malor *et al.* (1976) because we have shown that intraperitoneal THC is equi-active in obese and small mice.

A further advantage of oral over intraperitoneal dosing is that Tween 80 is inactive orally but produces abdominal constriction on intraperitoneal injection (Whittle, 1964) and thus the vehicle is active in the ring test with attendant complications (Table 1).

The results suggest that doses of oral THC in the mouse correlate closely with those used in clinical trials with synthetic THC orally in man. For example, (a) Noyes *et al.* (1976) gave 40 patients between 0.08 and 0.48 mg/kg of THC and found that all were sedated; (b) Graham *et al.* (1974) gave 6 patients 0.2 mg/kg of THC and found that all were drowsy and (c) Hollister *et al.* (1968) gave volunteers 0.6 mg/kg of THC orally and again recorded drowsiness. Our results also indicate that the time course for activity after oral THC is similar in mouse and man (Isbell *et al.*, 1967; Jones, 1971; Sallan *et al.*, 1975) for in both, peak activity is reached between 2 to 4 h.

Our results also show that THC has comparable cataleptic activity to chlorpromazine. For the first 2 h after dosing they are about equi-potent but the activity of THC continues to increase, whereas that of chlorpromazine begins to decline. At the peak of the THC activity it is 5.67 times as active as chlorpromazine (Table 5).

In the wild, mice which are voracious feeders on larval Lepidoptera, would receive an active dose of THC when they feed on larvae containing 0.07 mg THC (equivalent to about 3.5 mg/kg of mouse) which Rothschild, Rowan & Fairbairn (1974) have shown is the amount present in larvae which have fed on the leaves of *Cannabis sativa L.* (THC strain).

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