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Δ^9-Tetrahydrocannabinol and its metabolites in monkey brains

The metabolism of (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), the major active constituent of marihuana, has been studied primarily in peripheral tissues of animals and man. The major metabolite has been identified as 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC), a product of liver microsomal hydroxylation (Agurell, Nilsson & others, 1969, 1970; Ben-Zvi, Mechoulam & Burstein, 1970; Burstein, Menezes & others, 1970; Foltz, Fentiman & others, 1970; Lemberger, Silberstein & others, 1970; Nilsson, Agurell & others, 1970; Wall, Brine & others, 1970; Christensen, Freudenthal & others, 1971). Further hydroxylation of 11-OH-Δ^9-THC was shown *in vitro* to form 8,11-dihydroxy-Δ^9-tetrahydrocannabinol (8,11-$(OH)_2$-Δ^9-THC) (Wall & others, 1970; Christensen & others, 1971). The monohydroxylated metabolite has been reported to be behaviorally and pharmacologically as active as Δ^9-THC, whereas the dihydroxylated product is inactive (Wall & others, 1970; Truitt, 1970; Christensen & others, 1971).

In vitro incubation of mouse brain homogenate with ^{14}C-Δ^9-THC in the presence of a NADPH regenerating system did not produce an appreciable degree of hydroxylation (Christensen & others, 1971). In the only *in vivo* work thus far reported, both the 11-OH- and 8,11-$(OH)_2$-Δ^9-THC were found in brains of mice injected intravenously or intracerebrally with 0·2 mg of Δ^9-THC (Christensen & others, 1971).

The long retention of Δ^9-THC and its metabolites in rat brains (Ho, Fritchie & others, 1970; Layman & Milton, 1971), and the long-lasting behavioral effects of the compound in monkeys (Scheckel, Boff & others, 1968) prompted us to examine the metabolic alteration of Δ^9-THC in squirrel monkey brains at various times after its administration. Several thin-layer chromatography systems were developed to aid in the identification of Δ^9-THC and its metabolites.

Eight male squirrel monkeys (average 650 g) were injected intravenously *via* the saphenous vein with 10 or 1·5 mg/kg of ^{3}H-Δ^9-THC (146·3 μCi/mg) in 4% Tween 80 in saline. The animals were anaesthetized with ether and killed by bleeding at designated times. The brains were removed immediately, washed with saline and homogenized in four volumes of water. Triplicate aliquots (25 μl) of each homogenate were pipetted into counting vials; mixed with methanol then scintillation fluor, and assayed for tritium by liquid scintillation spectrometry. All values were corrected for 100% efficiency (channel ratio) and recovery.

For chromatography, each brain homogenate was extracted three times with five volumes of anhydrous methanol. The extracts were evaporated to dryness under nitrogen and the residue treated with a small volume of hot methanol. After centrifugation, the methanol solution was concentrated to less than one ml, and again centrifuged. The final supernatant, containing from 98 to 100% of the original radioactivity, was streaked on Silica Gel G precoated plates (Brinkmann Co.) and developed, along with reference compounds, in the solvent systems in Table 1. The distribution of radioactivity in the chromatograms was determined by scraping sections of silica (0·5 cm × 1 cm) and assaying for tritium. The percentage of each metabolite was calculated by a mapping technique which involved the plotting of per cent of radioactivity in each section *vs* R_F values.

Table 1. *% radioactivity recovered from brain, and its distribution (%) as unchanged Δ⁹-THC and metabolites after intravenous administration of ³H-Δ⁹-THC (10 mg/kg/i.v.) to squirrel monkeys.*

	% radioactivity** recovered from brain at			
	0·5 h	1 h	4 h	4 h†
	3·8	3·4	1·8	0·6
	4·4	2·6	1·7	0·8
	Proportions of recovered radioactivity* (%) recovered as Δ⁹-THC and its metabolites			
	0·5 h	1 h	4 h	4 h†
Δ⁹-THC	84	80	70	64
11-OH-Δ⁹-THC	8	10	15	33
8,11-$(OH)_2$-Δ⁹-THC ...	0	0	0	0
Unidentified‡	4·5	5	6	3

*Values were corrected for loss of radioactivity due to tritiated water.
**Individual values.
†1·5 mg/kg.
‡At the origin of chromatograms developed in the solvent systems B and C Table 2.

Table 2. *Chromatographic characteristics of Δ⁹-THC and derivatives.*

Compound	Solvent system* (R_F) A	B	C	D	E	Colour reaction†
Δ⁹-THC	0·70	0·59	0·94	0·96	0·90	Red
Δ⁸-THC	0·78	0·63	0·94	0·96	0·90	Red
Δ⁹,¹¹-THC	0·73	0·60	0·94	0·96	0·90	Red
Cannabinol	0·63	0·47	0·90	0·93	0·88	Purple
11-OH-Δ⁹-THC ...	0·03	0·11	0·59	0·78	0·39	Red
11-OH-Δ⁸-THC ...	0·03	0·11	0·59	0·79	0·39	Red
8,11-$(OH)_2$-Δ⁹-THC ...	0	0·01	0·14	0·41	0·09	Red

*Solvent systems on t.l.c.: A, n-hexane–acetone (10 : 1) then n-hexane–benzene (1 : 1); B, n-hexane–acetone (5 : 1); C, chloroform–acetone (4 : 1); D, chloroform–acetone (1 : 1); E, n-hexane–acetone–ether (3 : 1 : 1).
†Brentamine Fast Blue.

For enzymatic hydrolysis of possible conjugates, the eluants after evaporation were incubated at 37° overnight in sodium acetate buffer pH 5·0, with Sulfatase (aryl sulfatase from limpets, Sigma Chemical Co.).

Δ⁹-THC entered the brain rapidly with a maximum accumulation of 4% of the injected radioactivity at 30 min (Table 1), at which time the unchanged compound and the 11-hydroxylated metabolite accounted for 84 and 8% of the total radioactivity in the brain (Table 1). An average 28% decrease of radioactivity was observed from 30 min to one h, but the ratio of Δ⁹-THC and 11-OH-Δ⁹-THC was not vastly altered. At 4 h, 33% of the radioactivity found at 30 min still remained in the brain, while the percentage of hydroxylated metabolite increased and the unchanged compound decreased from the 1 h interval.

In contrast to the findings in mice reported by Christensen & others (1971), we did not detect any 8,11-dihydroxylated metabolite in the squirrel monkey brain. Table 2 shows the chromatographic characteristics of Δ⁹-THC and derivatives.

Besides the uhchanged and 11-OH-Δ⁹-TCH, a third area of radioactivity was present at the origin of chromatograms developed in both solvent system B and C (Table 2). When the eluant of the area was hydrolysed with Sulfatase, then chromotographed, an increase of approximately 1% radioactivity in the area corresponding to the R_F value

of Δ^9-THC indicated the existence of small amount of Δ^9-THC as the sulphate conjugate. The remaining material after hydrolysis showed a broad peak (R_F = 0·82) in an acidic solvent system of n-butanol–acetic acid–water (4 : 1 : 1), but a lower R_F (0·45) in a basic solvent system of n-butanol–2-propanol–ammonia–water (3 : 1 : 1) R_F (0·45) in a basic solvent system of n-butanol–2-propanol–ammonia–water (3 : 1 : 1 : 1). Neither of the two R_F values coincided with those of Δ^9-THC or other reference compounds listed in Table 2 using the same two solvent systems. Judging from the behaviour in acidic and basic solvents, this compound might possibly be an acid metabolite of Δ^9-THC; insufficient material was available for further characterization.

Δ^9-THC may undergo slow aromatization to cannabinol (Mechoulam, 1970). We did not detect any cannabinol from Δ^9-THC in monkey brains even when chromatograms were developed in the presence of light and air. Also we did not observe any isomerization of Δ^9-THC to the more stable Δ^8-THC isomer.

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