The uptake and metabolic fate of cannabinoids in rat brains

The major metabolite of (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC*), has been identified as 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC†), a product of liver microsomal oxidase reaction. Some reports indicate that this monohydroxylated metabolite is as active as Δ^9-THC behaviorally and pharmacologically (Irwin, 1968; Wall, Brine & others, 1970; Truitt, 1970; Gill & Jones, 1972), while others imply that unchanged Δ^9-THC is responsible for certain effects of marihuana (Sofia & Barry, 1970; Kubena & Barry, 1970). Lemberger, Crabtree & Rowe (1972) reported that 11-OH-Δ^9-THC and its metabolites, mostly as yet unidentified, are excreted in human urine and faeces for more than a week, while Gill, Jones & Lawrence (1973) also reported that 11-OH-Δ^9-THC, when administered to mice, was found to be mostly unchanged in the brain.

Δ^8-THC differs from its isomer Δ^9-THC only in the position of the double bond. Although the amount present in marihuana plant is much less than Δ^9-THC (Hively, Mosher & Hoffman, 1966), Δ^8-THC is also behaviorally and pharmacologically active. Ho, Taylor & others (1971), demonstrated the different effects of the two isomers on behaviour and brain amine levels in monkey and mouse brains, and believe that as well as a difference in potency between Δ^8-THC and Δ^9-THC there may also be a qualitatively different spectrum of psychopharmacological action. Segal & Kenney (1972) have substantiated these findings in cats using electroencephalographic responses. The aromatic cannabinol is present in marihuana plant in substantial quantity (Agurell & Leander, 1971). The general impression is that the compound is behaviorally inactive, but reconfirmation of this is necessary.

In conjunction with our previous report on the identification of Δ^9-THC and its metabolites in monkey brains (Ho, Estevez & others, 1972), we have examined four structurally related cannabinoids, Δ^9-THC, Δ^8-THC, 11-OH-Δ^8-THC, and cannabinol for comparison of their uptake and metabolic transformation in the rat brain.

Male Sprague-Dawley rats (175–200 g) were injected intravenously via the tail vein with 5 mg kg^{-1} of the following cannabinoids in Tween-80 and saline suspension: ^{3}H-Δ^9-THC (146·3 μCi mg^{-1}; 45·9 mCi $mmol^{-1}$), ^{3}H-Δ^8-THC (55·4 μCi mg^{-1}, 17·4 mCi $mmol^{-1}$), ^{3}H-11-OH-Δ^8-THC (29·3 μCi mg^{-1}, 9·67 mCi $mmol^{-1}$), or [^{3}H]cannabinol (72·4 μCi mg^{-1}, 22·4 mCi $mmol^{-1}$). The animals were decapitated at designated times after injections. Brains were washed in saline, homogenized with four volumes of water and extracted three times with five volumes of methanol. The extracts were evaporated to dryness under nitrogen, and the residue, redissolved in one ml of methanol, was chromatographed on Silica Gel precoated plates in the solvent system of chloroform–acetone (4:1). The distribution of radioactivity was determined by scraping sections of silica and assaying for tritium. The R_F values of the radioactive areas were then compared with the reference compounds.

For use as a reference standard, 11-OH-cannabinol was obtained by an *in vitro* incubation of cannabinol with rat liver supernatant from animals chronically treated with phenobarbitone to stimulate the activity of the microsomal enzyme system. Livers from two male rats pretreated four days with 75 mg kg^{-1} of sodium phenobarbitone twice daily were homogenized with 1·5 volumes of ice cold 1·15% KCl. The homogenate was centrifuged at 10 000 *g* for 10 min, and the supernatant was used as the enzyme source. A mixture consisting of 5·7 mg of [^{3}H]cannabinol in 0·1 ml of ethanol, 37 mg of nicotinamide, 3 mg of NADP, 180 mg of $MgCl_2 \cdot 6H_2O$, 30 ml of the liver supernatant, and 0·1M phosphate buffer, pH 7·0, in a final volume of 50 ml was incubated at 37° for 1 h. Unchanged cannabinol was extracted four times with five volumes of light petroleum (b.p. 30–60°); recovery 70%. The remaining radioactivity

* Also known as Δ^1- THC. † Also known as 7-hydroxy-Δ^1-THC.

Table 1. *Distribution of cannabinoids and metabolites in rat brain.*

Compound	15 min	n mol g^{-1} ± s.e. 30 min	60 min
^{3}H-Δ^9-THC	11·2 ± 0·05	10·9 ± 0·05	7·9 ± 0·05
^{3}H-Δ^8-THC	10·6 ± 0·02	11·5 ± 0·02	9·1 ± 0·03
	N.S.	N.S.	N.S.
^{3}H-11-OH-Δ^8-THC	10·8 ± 0·06	7·1 ± 0·03	5·8 ± 0·04
	N.S.	P <0·01	N.S.
[^{3}H]Cannabinol	8·3 ± 0·04	7·5 ± 0·01	6·0 ± 0·02
	P <0·01	P <0·001	P <0·02

Each value represents the mean (±s.e.) of four animals.
The P values represent the significance of differences between Δ^9-THC and other compounds. N.S.: not significant.

in the incubation mixture was recovered by extraction with diethyl ether. Chromatography of a fraction of this ether extract on silica gel plates with chloroform–acetone (4:1) as solvent showed the concentration of most of the radioactivity due to 11-OH-cannabinol at $R_F = 0{\cdot}48$. The ether extracts were then purified by the method of Widman Nilsson & others (1971). The structure of the isolated 11-OH-cannabinol was confirmed by comparison of several significant peaks in its mass spectrum with those previously reported for the compound [*m/e:* 326, M^+; 311, M^+-CH_3; 254, M^+-(CH_3+C_4H_9)].

For the determination of partition coefficients, a 1 mg sample of each tritiated compound was partitioned between equal volumes (10 ml) of water and benzene. Each tube was shaken for one h at ambient temperature, and was then allowed to stand until a clear separation of phases was reached (about 1 h). Triplicate aliquots of 25μl of each phase were assayed for tritium by liquid scintillation spectrometry. Partition coefficients were calculated as the ratio of radioactivity in the organic phase to that in the aqueous phase.

The distribution of the four labelled cannabinoids and metabolites in the rat brain at various time intervals is shown in Table 1. Since the specific activity varies with each compound, comparison was made of the number of n mol g^{-1} of brain tissue. At the early 15 min interval, the uptake of Δ^9-THC, Δ^8-THC, and 11-OH-Δ^8-THC and metabolites in the rat brain was to nearly the same extent. The concentration of cannabinol and metabolite, however, was lower than that of the three tetrahydrocannabinols.

Table 2 shows the amount of unchanged cannabinoids and their corresponding 11-hydroxylated metabolites at different times. After the intravenous administration of 11-OH-Δ^8-THC, practically no metabolite other than the unchanged compound was found in the brain. The extent of metabolism of the other three cannabinoids is reflected by the ratio of the unchanged compound to the 11-hydroxylated metabolites. The compounds found in rat brains are not due to blood contamination, since the

Table 2. *Metabolites in rat brain after administration of cannabinoids.*

Administered compound	15 min Unchanged compound	15 min Major metabolite[a]	15 min Ratio[b]	30 min Unchanged compound	30 min Major metabolite[a]	30 min Ratio[b]	60 min Unchanged compound	60 min Major metabolite[a]	60 min Ratio[b]
	nmol g^{-1} of brain								
Δ^9-THC	9·64	1·29	7·4	8·88	1·79	4·96	6·19	1·56	3·97
Δ^8-THC	9·40	1·05	8·95	9·29	1·85	5·02	6·71	2·28	2·94
11-OH-Δ^8-THC	10·38	0·03	346	6·80	0·03	227	5·46	0·02	273
Cannabinol	6·40	1·71	3·74	5·28	2·02	2·61	4·07	1·84	2·21

[a] 10,11-dihydroxylated compound from 11-OH-Δ^8-THC, and the corresponding 11-hydroxylated compounds from the other three cannabinoids.
[b] Ratio of the unchanged compound to the major metabolite.

corresponding dihydroxylated metabolites normally present in blood (Wall & others, 1970) in substantial amounts were not detected in the brain. At the two early intervals, the aromatic cannabinoid underwent more rapid transformation than the two tetrahydro analogues. When the ratio of unchanged to metabolite of the two tetrahydro congeners are compared, the higher value for Δ^8-THC at 15 min indicates the possibility of a lower initial rate of metabolism of the compound than that of Δ^9-THC. Since the microsomal fraction from rat brains does not metabolize Δ^9-THC to its 11-hydroxy derivative (Christensen, Freudenthal & others, 1971), it is assumed that the 11-hydroxylated metabolites of the cannabinoids were formed in the liver and then transported to the brain via blood circulation.

The only correlation found between the uptake of cannabinoids in the brain and the partition coefficients, was in the case of cannabinol where the compound having the least lipophilicity among the four shows the lowest brain level at early time intervals [Benzene–water partition coefficients (n = 9): Δ^9-THC 5880, Δ^8-THC 4760, 11-OH-Δ^8-THC 1450, cannabinol 1175]. A hydroxyl group on the tetrahydrocannabinol structure increases the polarity of the compound, as reflected by the increased partition coefficient between water and benzene. The 11-OH-Δ^8-THC, however, entered the brain in an amount equivalent to Δ^8-THC and Δ^9-THC. To account for this phenomenon factors other than lipophilicity must be involved and among them a weaker plasma binding of the 11-hydroxylated metabolite as compared to Δ^8-THC and Δ^9-THC is possible. This difference in plasma binding would make more unbound 11-OH-Δ^8-THC available to the brain. The benzene–water partition values described in this work are consistent with those reported for Δ^9-THC and 11-OH-Δ^9-THC using an n-octanol–water system (Gill & others, 1973).

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