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BRAIN MICROSOMAL OXIDATION OF Δ^8- AND Δ^9-TETRAHYDROCANNABINOL

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SUMMARY: Brain microsomes of mice, rats, guinea pigs and rabbits catalyzed the oxidation of Δ^8- and Δ^9-tetrahydrocannabinol to their monohydroxylated metabolites. The most prominent metabolite was the 4'-hydroxylated metabolite on the pentyl side chain of the cannabinoids in all species tested, except that the 5'-hydroxylation of Δ^9-tetrahydrocannabinol was most abundant in the guinea pig. These results are quite different from the metabolic profile of the cannabinoids with hepatic microsomes.

Several lines of evidence have demonstrated that brain microsomes contain cytochrome P 450-dependent mixed function oxidases resembling those in the liver (1-4). Although biological significance of the mixed function oxidase in the brain has not been established, the enzyme system may play an important role for regulating the activity and toxicity of centrally acting drugs.

Tetrahydrocannabinol (THC), a psychoactive component of marihuana, is extensively metabolized with hepatic microsomes of animals including human (5-7). However, limited attention has been directed to the metabolism of THC in brain. Christensen et al. (8) and Jones et al. (9) reported that no conversion of THC to metabolites was found with brain post mitochondrial fractions. The present study reexamined the brain microsomal metabolism of Δ^8- and Δ^9-THC using different species of animals.

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MATERIALS AND METHODS

NADP and glucose-6-phosphate were purchased from Boehringer Mannheim GmbH., and glucose-6-phosphate dehydrogenase (type V) and Fast Blue BB salt were from Sigma Chem. Co. Other chemicals used were best quality of commercially available. Δ^9-THC was purified from cannabis leaves as reported previously (10). Δ^8-THC, 7β-hydroxy-Δ^8-THC (7β-OH-Δ^8-THC), 4'-OH-Δ^8-THC and 11-OH-Δ^8-THC were prepared by the previous methods (11-14). 4'-OH- and 5'-OH-Δ^9-THC were supplied from National Institute on Drug Abuse. Male mice (ddN, 25-35 g), rats (Wistar, 200-350 g), guinea pigs (Hartley, 300-350 g) and rabbits (2.5-3.5 kg) were used. Microsomes were prepared as previously described (15). Incubations were carried out with microsomes equivalent to 10 to 15 g of brain, 5 μmol NADP, 50 μmol $MgCl_2$, 40 μmol nicotinamide, 5 μmol EDTA-2Na, 1.6 μmol Δ^8- or Δ^9-THC in dimethylsulfoxide, 500 μmol patassium phosphate buffer (pH 7.4) to make a final volume of 10 ml at 37 °C for 60 min. The metabolite formed were extracted twice with 20 ml of ethyl acetate. After the evaporation of the solvent, the residue was dissolved in a small amount of ethyl acetate and subjected to the preparative thin-layer chromatography (t.l.c.) with a solvent system of n-hexane-acetone-diethylamine (20:10:1). THC metabolites were detected by spraying 0.1% Fast Blue BB salt. The subsequent procedure for gas chromatography-mass spectrometric (GC-MS) analysis of THC metabolites was carried out by the previous method (7). GC-MS conditions were as follows: a JEOL GCG-06 gas chromatograph coupled with a JEOL JMS-DX 300 mass spectrometer and a JEOL JMS-DA 500 data system, column 5% SE-30 on Chromosorb W (60-80 mesh; 3 mm x 2 m), column temperature 250 °C, carrier gas He 40 ml/min, ionizing energy 70 eV and ionizing current 300 μA. The identification of THC metabolites was based on the comparison of their retention times in GC and mass spectra with those of authentic samples (7β-OH-Δ^8-THC, 4'-OH-Δ^8-THC, 11-OH-Δ^8-THC, 4'-OH-Δ^9-THC and 5'-OH-Δ^9-THC) and the data reported by others (5'-OH-Δ^8-THC)(16,17). The relative abundance of the metabolites identified was calculated from peak areas of the metabolites on the mass chromatogram.

RESULTS AND DISCUSSION

The t.l.c. analyses of ethyl acetate extracts of brain microsomes in all aniaml species incubated with Δ^8- or Δ^9-THC showed Fast Blue BB-positive spots mainly located with Rf values corresponding to authentic monohydroxylated metabolites of THCs. After the preparative t.l.c., the metabolites were extracted with ethyl acetate. The extracts were trimethylsilylated and then subjected to GC and GC-MS. Typical gas chromatograms and mass spectra of Δ^8- and Δ^9-THC metabolites formed with mouse brain microsomes are shown in Fig. 1. M-I of Δ^8-THC had a retention time of 7.9 min and the metabolite was identified as 2-TMS-4'-OH-Δ^8-THC by its mass spectrum [m/z 474 (M^+, 100 %), 391 (49%), 330 (36%) and 117 (22%)]. M-II of Δ^8-THC had a retention time of 10.2 min and was identified as 2-TMS-5'-OH-Δ^8-THC [m/z 474 (M^+, 100%), 391 (67%) and 330 (61%)]. These spectra and the retention times of GC were identical with those of the authentic sample. M-I of Δ^9-THC had a retention

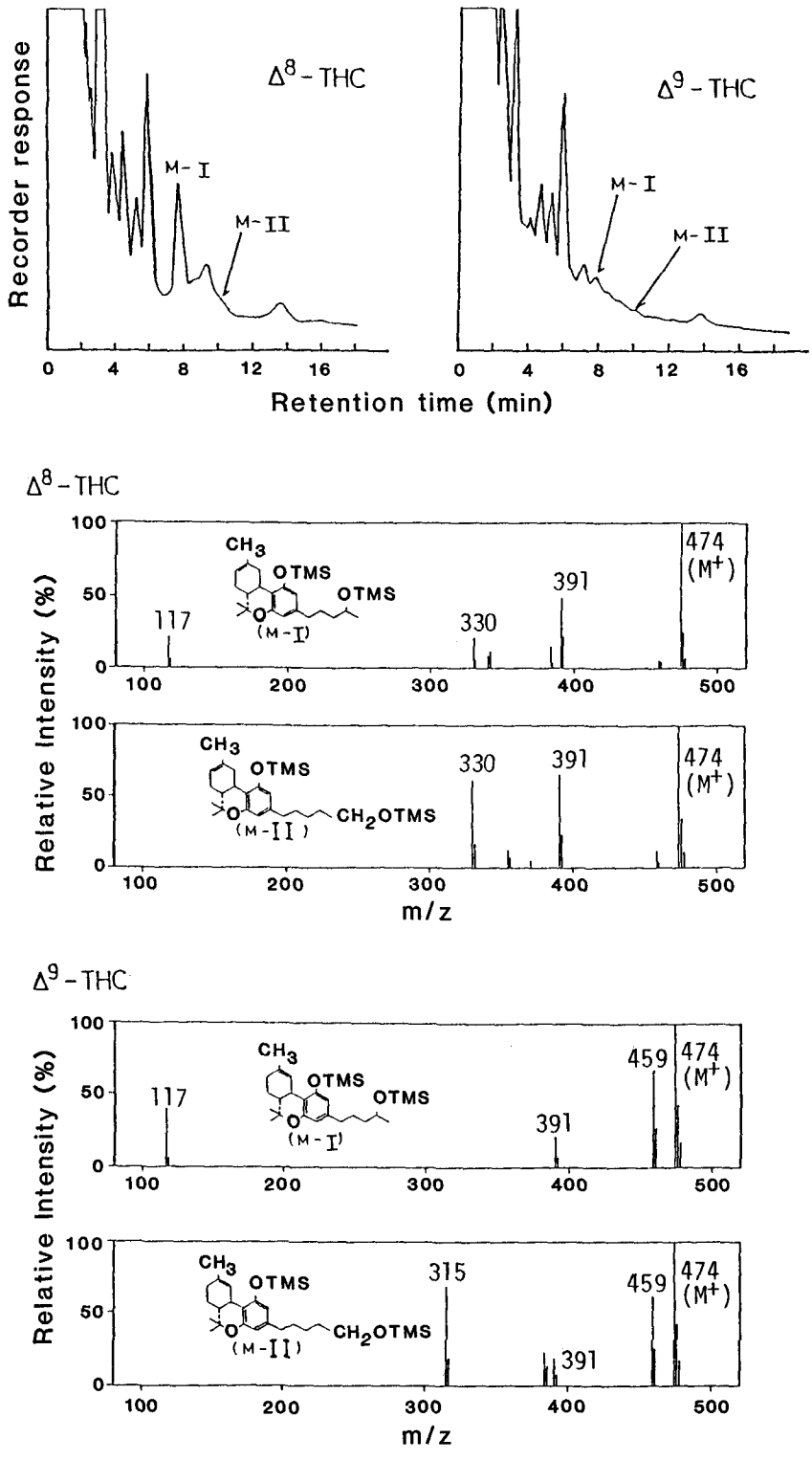

Fig. 1 Gas chromatogarms and mass spectra of TMS derivatives of Δ^8-THC and Δ^9-THC metabolites formed with mouse brain microsomes

time of 8.0 min and was identified as 2-TMS-4'-OH-Δ^9-THC [m/z 474 (M^+, 100 %), 459 (48%), 391 (19%), 330 (14%) and 117 (33%)]. M-II of Δ^9-THC had a retention time of 10.4 min. The metabolite was identified as 2-TMS-5'-OH-Δ^9-THC [m/z 474 (M^+, 100%), 459 (63%), 391 (22%) and 315 (70%)].

The experiments with rat, guinea pig and rabbit brain microsomes were carried out in the similar manner. The results are summarized in Table I. It revealed that 4'-OH-Δ^8-THC and 4'-OH-Δ^9-THC were the most abundant metabolites of THCs formed with brain microsomes of all animal species except for guinea pig. 5'-OH-Δ^9-THC was the most abundant metabolite of Δ^9-THC with guinea pig brain microsomes. 5'-OH-Δ^9-THC was also formed with mouse and rabbit brain microsomes to lesser extents. Other metabolites identified were 5'-OH-Δ^8-THC (rabbit), 11-OH-Δ^8-THC (rat, guinea pig and rabbit) and 7β-OH-Δ^8-THC (rat and guinea pig).

The present study clearly demonstrated that brain microsomes of mouse, rat, guinea pig and rabbit are capable to metabolize Δ^8- and Δ^9-THC to their monohydroxylated metabolites mainly on the pentyl side chain. Although it has been reported that mouse brain preparation could not metabolize Δ^9-THC (8,9), the discrepancy between the result of the present study and earlier reports may be due to the enzyme volume used in the both studies. The metabolic profiles of THCs in brain microsomes were quite different from those

Table I Relative peak areas on mass chromatogram of TMS derivatives of Δ^8-THC and Δ^9-THC formed with brain microsomes

Substrates	Metabolites	Mouse	Rat	Guinea pig	Rabbit
Δ^8-THC	4'-OH-Δ^8-THC	100	100	100	100
	5'-OH-Δ^8-THC	13	—[a)]	—	80
	11-OH-Δ^8-THC	—	40	64	78
	7β-OH-Δ^8-THC	—	31	88	—
Δ^9-THC	4'-OH-Δ^9-THC	100	100	82	100
	5'-OH-Δ^9-THC	60	—	100	25

a) not detected (10 <).

in hepatic microsomes (5-7), but relatively similar to those in lung (16, 18). 11-Hydroxylation is known to be the most predominant route of THC oxidation with hepatic microsomes of almost of animal species including human. In contrast, 4'-OH-Δ^9-THC has been reported to be the most abundant metabolite of Δ^9-THC by lung preparations. It is now clear that different forms of cytochrome P 450 are present in hepatic microsomes of animals (19, 20). The metabolic profile of drugs with microsomes is, therefore, dependent on the extent of specific forms of cytochrome P 450 in the microsomes. Recently, there have been several reports concerning the extent of cytochrome P 450 in brain microsomes (21,22), although none is known to what extent the specific forms of cytochrome P 450 are in the brain. The difference in the metabolic profiles of THCs between hepatic and brain microsomes seems to reflect the difference in the extent of the specific forms of cytochrome P 450.

In conclusion, THCs are hydroxylated mainly at the 4'-position with brain microsomes of animals to form 4'-OH-Δ^8-THC and 4'-OH-Δ^9-THC. Pharmacological significance of THC metabolism in the brain must be elucidated, because the metabolites idenfified are known to have some pharmacological activity (14,23).

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