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SEX DIFFERENCE IN THE OXIDATIVE METABOLISM OF Δ^9-TETRAHYDROCANNABINOL IN THE RAT

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Abstract—Oxidative metabolism of Δ^9-tetrahydrocannabinol (THC), one of the major components of marihuana, was studied using liver microsomes of adult male and female rats. There was no significant difference in the rates of the cannabinoid oxidation in terms of nmol per min per nmol of liver microsomal cytochrome P450 or of nmol per min per mg of microsomal protein between male and female rats. Δ^9-THC was biotransformed to various metabolites including 11-hydroxy-Δ^9-THC (11-OH-Δ^9-THC), 8α-OH-Δ^9-THC, 8α,11-diOH-Δ^9-THC, 3′-OH-Δ^9-THC by liver microsomes of male rats, while it was oxidized selectively to 11-OH-Δ^9-THC by liver microsomes of female rats. After intraperitoneal administration of Δ^9-THC, various metabolites were again found in the liver of the male rat, while in the female rat oxidation of the methyl group at the 9-position was a major metabolic pathway. These results demonstrate that an apparent sex-related difference exists in the oxidative metabolism of Δ^9-THC in the rat.

Δ^9-Tetrahydrocannabinol (THC) is the major psychoactive component of marihuana [1], and its metabolism has been extensively studied in various animal species, showing that oxidation of a methyl group at the 9-position is the common and major metabolic pathway of Δ^9-THC in almost all mammals examined [2].

The rat is one of the experimental animal species which have most frequently been used in the cannabinoid studies [3–15]. During a series of studies on the cannabinoid metabolism, we noticed that few studies on the *in vitro* metabolism of Δ^9-THC in the rat were carried out using female rats, although it is a typical animal species which exhibits sex differences in drug metabolism especially in the liver [16]. We thus conducted the present study to examine whether or not any sex differences exist between male and female rats for *in vitro* and *in vivo* Δ^9-THC metabolism (Fig. 1).

MATERIALS AND METHODS

Chemicals and biochemicals. Various chemicals and enzymes were obtained as follows: NADP and glucose-6-phosphate (G-6-P) were purchased from Boehringer-Mannheim GmbH (Darmstadt, F.R.G.); G-6-P dehydrogenase (type V, EC 1.1.1.49) and Fast Blue BB salt were from the Sigma Chemical Co. (St Louis, MO, U.S.A.); Sephadex LH-20 was from Pharmacia Fine Chemicals (Uppsala, Sweden); *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylsilylimidazole (TMSI) and trimethylchlorosilane (TMCS) were from Tokyo Kasei Kogyo Co. (Tokyo, Japan); precoated silicagel plates (SIL G-25 UV 254, 0.25 mm thickness × 20 cm) were from Macherey-Nagel (Duren, F.R.G.). Δ^9-THC was purified from cannabis leaves by the method reported previously [17]. 8α-Hydroxy-Δ^9-THC (8α-OH-Δ^9-THC), 8β-OH-Δ^9-THC [18], 11-oxo-Δ^9-THC [19] and 9α,10α-epoxyhexahydrocannabinol (EHHC) [20] were prepared by the reported methods. 11-OH-Δ^9-THC, Δ^9-THC-11-oic acid, 1′-OH-Δ^9-THC, 2′-OH-Δ^9-THC, 3′-OH-Δ^9-THC, 4′-OH-Δ^9-THC and 5′-OH-Δ^9-THC were supplied by National Institute on Drug Abuse (Bethesda, MD, U.S.A.). Purities of these cannabinoids were checked by gas chromatography (GC) to be in the range of 91 to 98%. Other chemicals used were of the best quality commercially available.

Animals. Adult male and female rats of Sprague–Dawley strain were purchased from Hokuriku Experimental Animals Lab. (Kanazawa, Japan). The animals were kept in an air-conditioned room (23–24° of the ambient temperature) with a 12 hr light cycle, and allowed to take food (Oriental MF-3 obtained from Oriental Yeast Co., Tokyo, Japan) and water *ad lib.*

In vitro *metabolism of* Δ^9-*THC.* Rats were killed by decapitation and livers were perfused with ice-cold physiological saline. The liver was homogenized in 4 volumes of 1.15% KCl containing 1 mM EDTA-2Na with a Teflon–glass homogenizer. The homogenate was centrifuged and microsomal suspension was prepared according to the method reported previously [21]. Reaction medium in a conical tube (10 mL of the volume) consisted of G-6-P (5 μmol), NADP (0.5 μmol), $MgCl_2$ (10 μmol), G-6-P dehydrogenase (1 I.U.), microsomes (equivalent to 0.1 g liver), and sodium–potassium phosphate buffer (100 mM, pH 7.4) to make a final volume of 1.0 mL. Incubation at 37° was started by adding Δ^9-THC (50 μg in 2 μL of dimethylsulfoxide, 159 μM), and continued for 30 min. Reaction was

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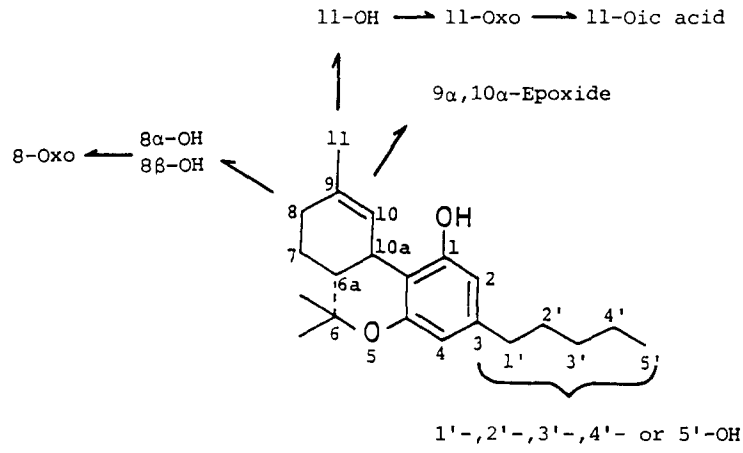

Fig. 1. Known oxidative metabolic pathways of Δ^9-THC.

terminated by adding ethyl acetate (EtOAc, 5 mL). After addition of 5α-cholestane (2 μg) as an internal standard to the aqueous layer, and the tube was vigorously shaken for 10 min and centrifuged. The upper organic layer was taken and evaporated *in vacuo*. The residue was dissolved in 40 μL of acetonitrile, and a portion (10 μL) was subjected to TLC using a precoated silica gel plate and *n*-hexane/acetone/diethylamine (20:10:1, v/v/v) as a solvent system. Metabolites were located by spraying 0.1% (w/v) aqueous solution of Fast Blue BB salt. The remaining sample was heated with BSTFA (8 μL), TMSI (4 μL) and TMCS (4 μL) at 60° for 10 min. The trimethylsilylated metabolites were analysed by GC and gas chromatography–mass spectrometry (GC/MS).

In time-course experiments, Δ^9-THC (50 μg) was incubated with microsomes under the conditions for the oxidative metabolism, and remaining substrate was determined without derivatization by GC on the basis of a calibration curve. For kinetic analysis, various amounts of Δ^9-THC (15.9–509.6 μM) was incubated with microsomes and an NADPH-generating system at 37° for 10 min. Metabolites were extracted as described above, and analysed by GC and GC/MS as trimethylsilyl (TMS) derivatives. Metabolite formation was analysed by Lineweaver–Burk or Hill plots [22].

Calibration curves of Δ^9-THC metabolites. Known amounts (25 ng–10.0 μg) of nine metabolites of Δ^9-THC (11-OH-Δ^9-THC, 8α-OH-Δ^9-THC, 8β-OH-Δ^9-THC, 9α,10α-EHHC, 1'-OH-Δ^9-THC, 2'-OH-Δ^9-THC, 3'-OH-Δ^9-THC, 4'-OH-Δ^9-THC and 5'-OH-Δ^9-THC) and 5α-cholestane (2 μg) were added to the ice-cold reaction medium containing the same ingredients as described above except for omitting the substrate. Then, EtOAc (5 mL) was added and shaken. After centrifugation, the organic layer was evaporated *in vacuo*, and the residue was dissolved in 30 μL of acetonitrile. The metabolites were trimethylsilylated and analysed by GC and GC/MS as described above. A mass chromatogram for each metabolite was drawn by scanning its characteristic fragment ion listed in Table 1. Calibration curves were made by plotting ratio of peak area of each metabolite to that of 5α-cholestane on the mass chromatogram versus the amount ratio. For Δ^9-THC, 5–50 μg of the cannabinoid and 2 μg of 5α-cholestane were added to the ice-cold reaction medium as described above, and extracted with EtOAc in the same manner. After evaporation of the organic solvent, the residue was dissolved in 30 μL of acetonitrile and an aliquot (2 μL) was subjected to GC. Recovery of Δ^9-THC was 95.2%.

In vivo *metabolism of Δ^9-THC.* Δ^9-THC was suspended in physiological saline containing 1% Tween 80 (40 mg/mL), and injected intraperitoneally (100 mg/kg) to adult male (about 270 g of body wt) and female (about 230 g of body wt) rats. After 1 hr, the rats were killed by decapitation, and livers were homogenized in 3 volumes of acetone with a Polytron (Kinematica GmbH, Luzern, Switzerland) for 1 min, followed by homogenization with a Teflon–glass homogenizer. The homogenate was vigorously shaken and centrifuged, and the supernatant was removed. The pellet was subjected to the extraction procedure with acetone twice more, and the combined supernatant was evaporated *in vacuo*. The residue was dissolved in 5 mL of chloroform ($CHCl_3$), and applied to a column of Sephadex LH-20 equilibrated with $CHCl_3$. Metabolites were fractionated by increasing methanol concentration from 0 to 16% (v/v) in $CHCl_3$ according to the method reported by Harvey *et al.* [23]. Δ^9-THC metabolites in effluents were analysed by TLC, GC and GC/MS as described above. When synthetic standards were not available, trimethylsilylated metabolites were tentatively identified by comparing their fragment ions with those reported by Harvey [24].

Conditions for GC and GC/MS were as follows. GC: instrument, a Shimadzu GC-16A gas chromatograph equipped with a hydrogen flame ionization detector and a C-R5A Chromatopac data processor; column, 3% SE-30 on Chromosorb W (60–80 mesh, 3 mm i.d. × 2 m); column temperature, 260°; detector temperature, 275°; carrier gas, N_2

Table 1. Characteristics of Δ^9-THC metabolites in TLC, GC and GC/MS

Metabolites	Relative* R_f value	Relative retention time (min)†	Characteristic ion (m/z)	Recovery (%)
1'-OH-Δ^9-THC	0.58	0.355	417	91.1
9α,10α-EHHC	0.85	0.387	402	90.9
2'-OH-Δ^9-THC	0.62	0.446	145	93.8
8α-OH-Δ^9-THC	0.58	0.530	384	95.0
3'-OH-Δ^9-THC	0.55	0.547	330	94.8
8β-OH-Δ^9-THC	0.71	0.551	343	94.4
4'-OH-Δ^9-THC	0.52	0.555	474	92.7
11-OH-Δ^9-THC	0.53	0.566	371	93.1
5'-OH-Δ^9-THC	0.50	0.710	474	94.5

* Values are ratios to an R_f value (0.658) of Δ^9-THC in silica gel TLC (*n*-hexane/acetone/diethylamine = 20:10:1 as a solvent).

† Values are ratios to a retention time (11.38 min) of 5α-cholestane on a 3% SE-30 (3 mm × 2 m) column. Other conditions in GC and GC/MS were described in Materials and Methods.

50 mL/min; injection volume, 2 μL. GC/MS: instrument, a JEOL JMS DX-300 mass spectrometer equipped with a GCG-06 gas chromatograph and a JMA DA-5000 mass data system; column, 3% SE-30 on Chromosorb W (60–80 mesh, 3 mm i.d. × 2 m); column temperature, 260°; injection port temperature, 275°; carrier gas, He 40 mL/min; ionization energy, 70 eV; ionization current, 300 μA.

Protein concentration was determined by the method of Lowry *et al.* [25] using bovine serum

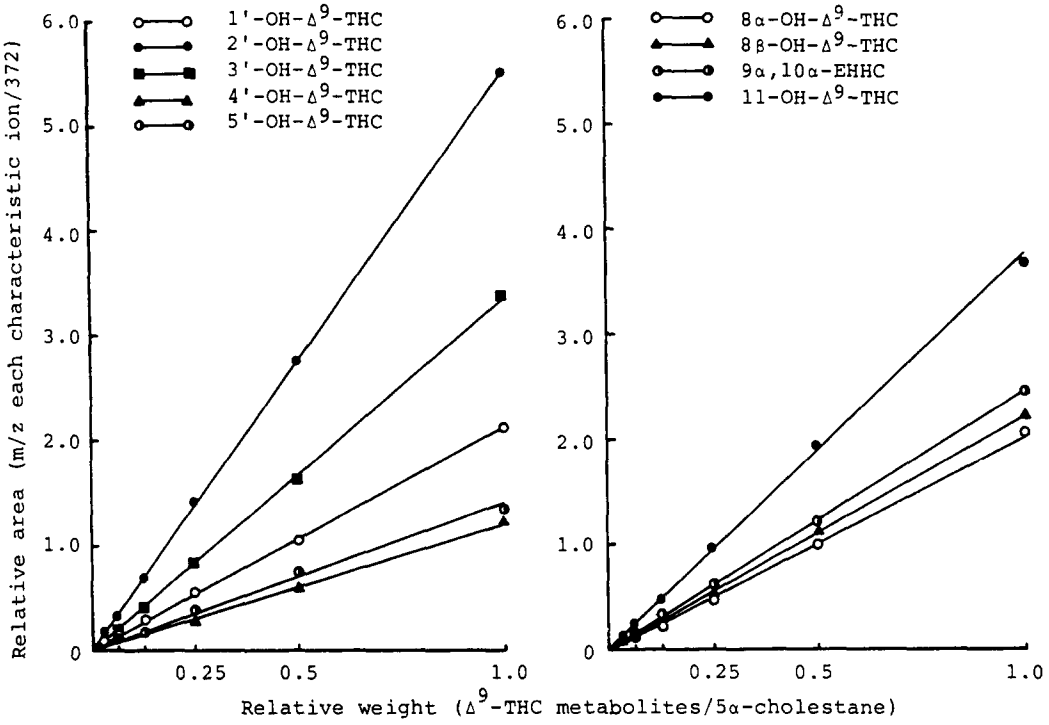

Fig. 2. Calibration curves for determination of Δ^9-THC metabolites. Various amounts of Δ^9-THC metabolites were added to the reaction medium containing the same ingredients as used in the microsomal reaction except for omitting the substrate, and extracted with EtOAc after adding internal standard. Metabolites were converted into TMS derivatives, and subjected to GC/MS under the conditions described in Materials and Methods. Fragment ion characteristic for each metabolite listed in Table 1 was scanned, and relative weights of Δ^9-THC metabolites to that of 5α-cholestane added to the medium were plotted versus relative areas of the metabolite peaks to that of the internal standard on the mass chromatograms.

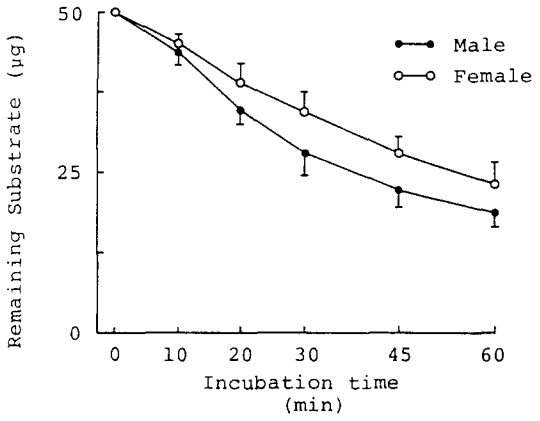

Fig. 3. Comparison of Δ^9-THC oxidation by liver microsomes of male and female rats. Δ^9-THC (50 μg) was incubated at 37° in the reaction medium consisting of G-6-P (5 μmol), NADP (0.5 μmol), $MgCl_2$ (10 μmol), G-6-P dehydrogenase (1 I.U.), microsomes (0.1 g liver equivalent) and sodium–potassium phosphate buffer (100 μmol, pH 7.4). The substrate remaining in the mixture was determined by GC. Each point represents the mean value ± SE of three determinations where three different liver microsomes of each sex were used. Male (●); female (○).

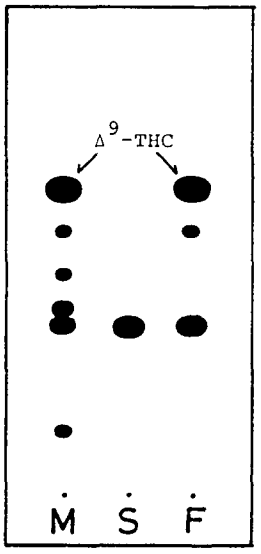

Fig. 4. A TLC profile of Δ^9-THC metabolites formed by liver microsomes of male and female rats. Δ^9-THC (50 μg) was incubated with liver microsomes (equivalent to 0.1 g liver) in the presence of an NADPH-generating system at 37° for 30 min. Metabolites formed were extracted with EtOAc, and a portion of the extract was submitted to silicagel TLC. A solvent system, *n*-hexane/acetone/diethylamine (20:10:1, v/v/v); a coloring reagent, 0.1% (w/v) aqueous solution of Fast Blue BB salt. The substrate (Δ^9-THC) was pointed out with arrows. S, synthetic 11-OH-Δ^9-THC; M, male; F, female.

albumin as the standard. Cytochrome P450 content was determined by the carbon monoxide difference spectrum with a Union SM 401 spectrophotometer ($\varepsilon = 91\ mM^{-1}$) [26]. Statistical significance of difference was calculated by Student's *t*-test.

RESULTS

Determination procedure of Δ^9-THC metabolites

Characteristics of nine Δ^9-THC metabolites in TLC, GC and GC/MS are listed in Table 1. Since most of trimethylsilylated metabolite peaks were eluted from 0.5 to 0.6 of relative retention times against 5α-cholestane in GC, they were determined by mass chromatographic techniques drawing their characteristic fragment ions (Table 1). Calibration curves are shown in Fig. 2. Fragment ions at *m/z* 117 and 315 were diagnostic ions for 2TMS-4′-OH-Δ^9-THC and 2TMS-5′-OH-Δ^9-THC, respectively, but their intensities in GC/MS were rather low. Therefore, these two metabolites were determined on the basis of their retention times and calibration curves made by scanning their molecular ions at *m/z* 474. Δ^9-THC was determined without derivatization by GC on the basis of its calibration curve (not shown). Recoveries of Δ^9-THC and its metabolites were in the range of 90.9 to 95.2%.

In vivo *metabolism of Δ^9-THC*

Δ^9-THC was incubated with liver microsomes of male or female rats in the presence of an NADPH-generating system. Amounts of Δ^9-THC remaining in the reaction medium at various time intervals of the incubation were determined by GC. Figure 3 shows the time course of Δ^9-THC metabolism. After 60 min of the incubation, 36.7 and 46.0% of the substrate added were metabolized with liver microsomes of male and female rats, respectively. However, there was not a significant difference in the rates of Δ^9-THC metabolism in terms of nmol per min per nmol of cytochrome P450 between male (2.28 ± 0.23) and female (2.57 ± 0.67) rats. On the basis of nmol per min per mg of protein, no significant difference was seen in the rates of the cannabinoid metabolism. A TLC profile of Δ^9-THC metabolites after 30 min incubation with microsomes under oxidation conditions is shown in Fig. 4. Five metabolite spots were seen on the chromatogram of the extract from the incubation mixture with liver microsomes of male rats. Among these spots, the major one had an R_f value of 0.34 corresponding to that of synthetic 11-OH-Δ^9-THC. On the other hand, there were only two spots on the chromatogram for the female rat liver microsomes. The major metabolite spot showed a similar R_f value to that of the synthetic 11-OH-Δ^9-THC. Remaining metabolite samples were then converted to TMS derivatives and analysed by GC and GC/MS (Table 2). The most abundant metabolite formed with male rat liver microsomes was 11-OH-Δ^9-THC, followed by 8α-OH-Δ^9-THC, 3′-OH-Δ^9-THC, 9α,10α-EHHC, 2′-OH-Δ^9-THC and 8β-OH-Δ^9-THC. For females, the most abundant metabolite was also 11-OH-Δ^9-THC followed by 9α,10α-EHHC. Amounts of 8α-OH-Δ^9-THC and 3′-OH-Δ^9-THC were very small.

Kinetic analysis

To analyse oxidative metabolism, Δ^9-THC in

Table 2. Oxidative metabolism of Δ^9-THC with liver microsomes of male and female rat

Sex	Metabolites	Activity (nmol/min/mg of protein)	
Male	11-OH-Δ^9-THC	1.53	(100)*
	8α-OH-Δ^9-THC	0.32	(21)
	3′-OH-Δ^9-THC	0.17	(11)
	9α,10α-EHHC	0.12	(8)
	8α,11-diOH-Δ^9-THC†	0.10	(7)
	8β-OH-Δ^9-THC	0.08	(5)
	2′-OH-Δ^9-THC	0.02	(1)
Female	11-OH-Δ^9-THC	1.92	(100)
	9α,10α-EHHC	0.21	(11)
	8β-OH-Δ^9-THC	0.04	(2)
	3′-OH-Δ^9-THC	0.02	(1)

Δ^9-THC was incubated with liver microsomes fortified with an NADPH-generating system at 37° for 30 min. Metabolites were extracted with EtOAc, and analysed by GC/MS as described in Materials and Methods. Values represent the mean of two determinations where two different liver microsomes for each sex were used.

* Relative activity (%).

† Since a synthetic standard was not available, this metabolite was identified by referring the data of Harvey [24]. The activity was tentatively calculated by using a calibration curve for 8α-OH-Δ^9-THC.

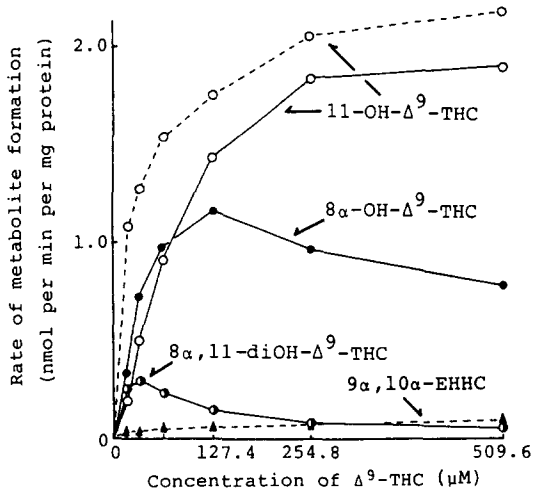

Fig. 5. Effects of Δ^9-THC concentration on the metabolism by liver microsomes of male and female rats. Δ^9-THC (15.9–509.6 μM) was incubated with liver microsomes in the presence of an NADPH-generating system at 37° for 10 min. Metabolites extracted with EtOAc were determined by GC/MS as TMS derivatives. Solid and broken lines represent male and female, respectively. Each point represents the mean value of two determinations where two different liver microsomes of each sex were used. 11-OH-Δ^9-THC (○); 8α-OH-Δ^9-THC (●); 8α,11-diOH-Δ^9-THC (◑); 9α,10α-EHHC (▲).

various concentrations was incubated with the liver microsomes of both sexes at 37° for 10 min. Metabolites were analysed by GC and GC/MS after conversion to TMS derivatives. Figure 5 shows the formation of major metabolites from Δ^9-THC for 10 min incubation at various concentrations of the substrate. For the male rats, the most abundant metabolite was 8α-OH-Δ^9-THC in the low concentrations of the substrate (to 63.7 μM). However, 11-OH-Δ^9-THC formation was increased concomitantly with increase of the substrate concentration, and it became the major metabolite in the concentration range of 127.4 to 509.6 μM of Δ^9-THC. In addition, 8β-OH-Δ^9-THC, 8-oxo-Δ^9-THC and 9α,10α-EHHC were also found to be formed, though their amounts were rather small comparing those of 11-OH-Δ^9-THC and 8α-OH-Δ^9-THC.

For the female rats, 11-OH-Δ^9-THC was the major metabolite at all the substrate concentrations employed. Further, the secondary abundant metabolite in female rats was 9α,10α-EHHC, followed by 8α-OH-Δ^9-THC and 8β-OH-Δ^9-THC. The metabolite formations by liver microsomes of female rats fitted Lineweaver–Burk plotting well. On the contrary, the profile of metabolite formation from Δ^9-THC with liver microsomes of male rats did not fit the Lineweaver–Burk plot but did fit the Hill plot. Kinetic parameters calculated are listed in Table 3.

In vivo *metabolism of* Δ^9-*THC*

Table 4 summarizes metabolites identified in the liver of male or female rats pretreated with Δ^9-THC and their relative abundance. In the male rat, the most abundant metabolite was 11-OH-Δ^9-THC followed by 8α,11-diOH-Δ^9-THC, 8α-OH-Δ^9-THC, 8β-OH-Δ^9-THC and 3′-OH-Δ^9-THC. In the female rat, 11-OH-Δ^9-THC was the major metabolite, and the amount of Δ^9-THC-11-oic acid found was one-fifth that of the major metabolite. A dihydrodiol metabolite was identified in a small amount, although epoxy metabolite was not identified under the conditions used. Its fragment ions were as follows; m/z 564 (M^+, 4%), 459 (base ion, 100%), 444 (42%), 385 (44%). Furthermore, there appeared a

Table 3. Kinetic parameters for oxidative metabolite formation from Δ^9-THC with liver microsomes of male and female rats

Metabolites	Sex	K_m(μM)	V_{max} (nmol/min/mg protein)	Hill coefficients
11-OH-Δ^9-THC	Male	54.0 ± 7.1	2.04 ± 0.15	1.54 ± 0.11
	Female	16.8 ± 2.0	1.84 ± 0.17	
8α-OH-Δ^9-THC	Male	39.0 ± 4.7	1.13 ± 0.16	1.75 ± 0.08
	Female	45.3 ± 8.3	0.05 ± 0.01	
9α,10α-EHHC	Male	262.8 ± 54.1	0.06 ± 0.01	1.49 ± 0.07
	Female	201.1 ± 31.2	0.12 ± 0.01	

Metabolite formation was analysed by a Hill plot for male rats, and by a Lineweaver–Burk plot for female rats. Each value represents the mean ± SE of three determinations where three different liver microsomes of each sex were used.

Table 4. Oxidation metabolites of Δ^9-THC identified in the livers of male and female rats treated with Δ^9-THC

Sex	Metabolites	Relative abundance (%)*
Male	11-OH-Δ^9-THC	100
	8α,11-diOH-Δ^9-THC†	75
	8α-OH-Δ^9-THC	55
	8β-OH-Δ^9-THC	8
	3′-OH-Δ^9-THC	2
	11-Oxo-Δ^9-THC	1
Female	11-OH-Δ^9-THC	100
	Δ^9-THC-11-oic acid	16
	8α,11-diOH-Δ^9-THC†	8
	11,2′-diOH-Δ^9-THC†	1

A rat of each sex was intraperitoneally given Δ^9-THC (100 mg/kg), and killed 1 hr later. Metabolites in the liver were extracted with acetone, and fractionated by column chromatography using Sephadex LH-20. They were analysed by GC/MS as TMS derivatives under the conditions described in Materials and Methods.

* Values were calculated on the basis of peak areas of mass chromatograms in GC/MS.

† Tentatively identified by referring to the data of Harvey [24].

metabolite whose fragment ions were m/z 474 (M^+, 3%), 391 (13%), 130 (86%) and 108 (base ion, 100%).

DISCUSSION

The rat is one of animal species most frequently used in the various animal experiments of cannabinoids including metabolic studies [27]. However, most of these studies were carried out using male rats, and there were few reports on the sex difference in cannabinoid metabolism. Burstein and Kupfer [8] reported that liver microsomes of male rats metabolized Δ^9-THC faster than those of female rats. Consonant with them, Δ^9-THC appeared to be eliminated with liver microsomes from male rats more quickly than with those from female rats in the present study. However, there was no significant difference in the rate of Δ^9-THC metabolism in terms of nmol per min per mg of protein or nmol per min per nmol of cytochrome P450 between male and female rats.

Interestingly, a marked sex difference was seen in the profile of the oxidative metabolism of the cannabinoid *in vitro*. Namely, Δ^9-THC was biotransformed to various hydroxylated metabolites such as 11-OH-Δ^9-THC, 8α-OH-Δ^9-THC, 3′-OH-Δ^9-THC, 2′-OH-Δ^9-THC, 8β-OH-Δ^9-THC and 9α,10α-EHHC with microsomes from male rats. On the contrary, the microsomes from female rats could convert the cannabinoid into practically two metabolites such as 11-OH-Δ^9-THC and 9α,10α-EHHC. A similar tendency was observed in Δ^9-THC metabolism *in vivo*. That is, analysis of the oxidative metabolites in the liver of rats treated with the cannabinoid revealed that Δ^9-THC was oxidized at various positions in male rats, while oxidation of the methyl group at the 9-position was the major pathway in female rats. Further, 9α,10α-EHHC was found to be one of the major metabolites of Δ^9-THC in female rats *in vitro*, but it could not be identified as an *in vivo* metabolite either in male or female rats under the conditions used. Instead, a dihydrodiol metabolite was tentatively identified in the female rat liver in the present study. This metabolite is thought to be a hydrolytic product of 9α,10α-EHHC. Moreover, we found a metabolite whose characteristic ions [m/z 474 (M^+, 3%), 130 (86%) and 108 (100%)] were very similar to those of cannabielsoin that is thought to be formed biologically from cannabidiol (CBD) via epoxide [28, 29]. This metabolite was not found in the male rat either *in vivo* or *in vitro*. In addition, none of the other metabolites of CBD was identified in the present study. Recently, Harvey and Mechoulam [30] have reported that Δ^9-THC was found in the urine of humans administered with CBD. Therefore, there is a possibility of converse formation of CBD from Δ^9-THC in female rats *in vivo*. Another possibility is the formation of an elsoin type metabolite from the epoxide of Δ^9-THC. We are investigating these possibilities.

The kinetic analysis has also demonstrated a remarkable difference in the Δ^9-THC metabolism between male and female rats. Namely, the formation of the major metabolite, 11-OH-Δ^9-THC, and of

9α,10α-EHHC with liver microsomes from female rats could adequately be analysed by a Lineweaver–Burk plot. On the other hand, the data for THC metabolites formed with microsomes of male rat liver fitted the Hill plot instead of the Lineweaver–Burk plot.

These results indicate that a positive co-operativity exists in the interaction of cytochrome P450s with Δ^9-THC in liver microsomes of male rats. Hill coefficients calculated ranged from 1 to 2. We chose liver microsomes of the rat as the enzyme source in the present study. Liver microsomes of rats are known to contain a number of isozymes of cytochrome P450 [31]. Therefore, it is feasible that several isozymes oxidize the same positions of Δ^9-THC. In fact, we have recently revealed that both P450 UT-2 belonging to IIC6 of a gene subfamily [31], and UT-4 (IIA2) purified from liver microsomes of adult male rats can convert Δ^9-THC into 11-OH-Δ^9-THC [32]. Considering the data obtained from female rats fitted the double reciprocal plot, the system in microsomes of female rats may be more simple than that of male rats.

The results obtained here demonstrated significant differences in oxidative metabolism of Δ^9-THC between male and female rats. Cohn *et al.* [33] observed sex difference in behavior response to marihuana extract distillate (MED). They reported that female rats were more sensitive to the MED than male rats [33]. We showed in the present study that Δ^9-THC was mainly converted to 11-OH-Δ^9-THC and 9α,10α-EHHC in female rats. These are known to be active metabolites whose pharmacological activities are much higher than those of the parent compound in various pharmacological indices [34–36]. Therefore, the higher sensitivity in female rats than in male ones to MED may be due, at least in part, to the difference in the rate of active metabolite formations between male and female rats. In addition, 9α,10α-EHHC was reported as one of the oxidative metabolites in male rats [14]. Hence, this is the first report that demonstrates the epoxide formation in the female rat. The sex differences observed in the present study would be due to the different forms of cytochrome P450 contained in liver microsomes of male and female rats. We are now studying oxidative metabolism of various cannabinoids using cytochrome P450 isozymes purified from liver microsomes of male and female rats.

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