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EFFECT OF REPEATED ADMINISTRATION OF 11-HYDROXY-Δ^8-TETRAHYDROCANNABINOL, AN ACTIVE METABOLITE OF Δ^8-TETRAHYDROCANNABINOL, ON THE HEPATIC MICROSOMAL DRUG-METABOLIZING ENZYME SYSTEM OF MICE

KAZUHITO WATANABE, MAYUMI ARAI, SHIZUO NARIMATSU, IKUO YAMAMOTO* and HIDETOSHI YOSHIMURA†

Department of Hygienic Chemistry, School of Pharmacy, Hokuriku University, Kanazawa 920-11, Japan; and †Department of Hygienic and Forensic Chemistry, Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka 812, Japan

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Abstract—The effects of Δ^8-tetrahydrocannabinol (Δ^8-THC) and its major and active metabolite, 11-hydroxy-Δ^8-tetrahydrocannabinol (11-OH-Δ^8-THC), on the hepatic microsomal drug-metabolizing enzyme system were studied in mice. The repeated administration of 11-OH-Δ^8-THC (5 mg/kg/day, i.v.) for 3 or 7 days increased significantly the activities of aniline hydroxylase and *p*-nitroanisole *O*-demethylase. By the same treatment, cytochrome P-450 content (3 days) or NADPH–cytochrome *c* reductase activity (7 days) was also increased significantly. The treatment with Δ^8-THC for 7 days (5 mg/kg/day, i.v.) significantly increased aniline hydroxylase only. 11-OH-Δ^8-THC increased the V_{max}, but not the K_m, values for both drug-metabolizing enzymes, whereas Δ^8-THC decreased significantly the K_m value (270 μM) for *p*-nitroanisole *O*-demethylase as compared with the control (398 μM). Repeated administration of these cannabinoids for 7 days also increased the metabolism of Δ^8-THC by hepatic microsomes; this was attributed to an enhanced formation of 11-OH-Δ^8-THC. In contrast, microsomal formation of 7α-OH-Δ^8-THC was decreased significantly by treatment with Δ^8-THC. 11-OH-Δ^8-THC, but not Δ^8-THC, treatment increased the metabolism of 11-OH-Δ^8-THC by hepatic microsomes. These findings indicate that Δ^8-THC and 11-OH-Δ^8-THC treatment can induce hepatic microsomal drug-metabolizing enzymes and affect differently the catalytic properties of the enzymes.

Tolerance development to many pharmacological effects of Δ^8-tetrahydrocannabinol (Δ^8-THC) and Δ^9-THC, which are the active constituents of marihuana, is well established in both experimental animals and humans [1, 2]. It is not clear, however, whether the mechanism of the tolerance is metabolic or functional in origin. Lemberger *et al.* [3] reported for the first time that repeated consumption of marihuana causes a shorter plasma half-life of Δ^9-THC in humans. The hypothesis thus indicated was that repeated consumption of marihuana results in more rapid metabolism of Δ^9-THC. In connection with this hypothesis, Davis and Borgen [4] reported data that suggested a metabolic mechanism in the development of tolerance to Δ^9-THC in rats. Magour *et al.* [5] also reported that an acceleration in the conversion of Δ^9-THC to polar metabolites in brain may be responsible for the development of tolerance to Δ^9-THC. However, limited information is available concerning an increase in hepatic microsomal metabolism of drugs, including cannabinoids, after repeated administration of Δ^8-THC, Δ^9-THC, or cannabis extract. Induction of hepatic and pulmonary benzo[*a*]pyrene hydroxylase has been reported in rats after an acute high dose of Δ^8-THC, Δ^9-THC, or cannabis extract [6, 7]. Ho *et al.* [8] reported that chronic administration of Δ^9-THC leads to an increase in the metabolism of Δ^9-THC in the hepatic microsomal 10,000 *g* supernatant fraction of rats.

In contrast to the above findings, there are many conflicting reports indicating a lack of correlation between the development of tolerance and a change in the metabolic disposition of THC [9–15]. The conflicting data reported appear to reflect variations in dose, species, periods or routes of administration. Recently, we reported that repeated administration of Δ^8-THC or 11-OH-Δ^8-THC, a major and active metabolite of Δ^8-THC, caused development of tolerance to their hypothermic, cataleptogenic and pentobarbital-induced sleep-prolonging effects in mice, and that the magnitude of tolerance to 11-OH-Δ^8-THC was greater than that to Δ^8-THC [16–18]. The present study examines the effects of repeated administration of Δ^8-THC and 11-OH-Δ^8-THC on hepatic microsomal drug-metabolizing enzymes of mice at the time of tolerance development. We also investigated the effects of these cannabinoids on their own metabolism by hepatic microsomes.

MATERIALS AND METHODS

Animals. Male ddN mice weighing 20–25 g were used throughout the experiments. They were given food and water *ad lib.*

* Address all correspondence to: Dr. Ikuo Yamamoto, Department of Hygienic Chemistry, Hokuriku University, School of Pharmacy, 3, Ho Kanagawa-machi, Kanazawa 920-11, Japan.

Table 1. Change in relative liver weight in mice following repeated administration of Δ^8-THC or 11-OH-Δ^8-THC

Treatment	Relative liver weight (g/kg body weight)		
	1 day	3 days	7 days
Control	59 ± 1	56 ± 1	60 ± 2
Δ^8-THC	55 ± 2*	53 ± 2	53 ± 3
11-OH-Δ^8-THC	54 ± 1†	52 ± 1	52 ± 2*

Data represent the mean ±S.E. of five or six pairs from ten to twelve mice.
* Significantly different from the control ($P < 0.05$).
† Significantly different from the control ($P < 0.01$).

Chemicals. Δ^8-THC, 7α-OH-Δ^8-THC, 8α,9α-epoxyhexahydrocannabinol (EHHC) and 11-OH-Δ^8-THC were prepared by methods described previously [19–21]. Δ^8-THC and 11-OH-Δ^8-THC were suspended in saline containing 1% (v/v) Tween 80 and injected intravenously at a dose of 5 mg/kg. The control group was given an equivalent volume (0.1 ml/10 g body wt) of the vehicle. NADP and glucose-6-phosphate were purchased from Boehringer Mannheim Gbm. NADPH, cytochrome *c* (type III) and glucose-6-phosphate dehydrogenase (type V) were purchased from the Sigma Chemical Co. *N*, *O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and trimethylsilylimidazole (TMSI) were obtained from Tokyo Chemical Ind. Trimethylchlorosilane (TMCS) was obtained from Wako Pure Chemical Ind. All other chemicals used were of the best quality commercially available.

General procedures. Animals were killed by cervical dislocation and livers were perfused by cold 0.9% (w/v) NaCl to remove blood. The livers were excised, and 20% (w/v) homogenates were prepared with 1.15% (w/v) KCl. The subsequent procedures were carried out at 0–4°. The homogenate was centrifuged at 9,000 *g* for 20 min. The supernatant fraction was centrifuged at 105,000*g* for 1 hr. The resulting pellet was resuspended in 1.15% KCl solution and used as the enzyme source. Cytochrome P-450 and b_5 contents were determined by the methods of Omura and Sato [22]. Molar extinction coefficients of cytochrome P-450 and b_5 used were 91 and 185 mM^{-1} respectively. Cytochrome *c* reductase activity was measured by the method of Phillips and Langdon [23]. Microsomal protein concentration was determined by the method of Lowry *et al.* [24] using crystalline bovine serum albumin as a standard.

Aniline hydroxylase. The incubation mixture consisted of Tris–HCl buffer, pH 7.4 (100 mM), $MgCl_2$ (10 mM), NADP (0.5 mM), glucose-6-phosphate (10 mM), glucose-6-phosphate dehydrogenase (1 unit), aniline hydrochloride (1 mM) and 0.2 ml of microsomal suspension (0.2 g liver equivalent) in a final volume of 2 ml. The mixture was incubated at 37° for 10 min after addition of NADP. The reaction was terminated by addition of 1 ml of 20% (w/v) trichloracetic acid. After centrifugation, *p*-aminophenol formed was determined by the method of Imai *et al.* [25].

p-*Nitroanisole O-demethylase.* The incubation mixture consisted of potassium phosphate buffer, pH 7.4 (100 mM), $MgCl_2$ (10 mM), nicotinamide (4 mM), NADP (0.5 mM), glucose-6-phosphate (10 mM), glucose-6-phosphate dehydrogenase (1 unit), *p*-nitroanisole (1.5 mM) and 0.2 ml of microsomal suspension in a final volume of 2 ml. The mixture was incubated at 37° for 10 min, and the reaction was terminated by addition of 4 ml of 0.2 N trichloracetic acid. After centrifugation, the *p*-nitrophenol formed was determined by the method of Kato and Gillette [26].

Cannabinoid metabolism. Cannabinoids (Δ^8-THC, 500 μg; 11-OH-Δ^8-THC, 250 μg) were added

Table 2. Change in kinetic parameters of hepatic microsomal drug-metabolizing enzymes by repeated administration of Δ^8-THC or 11-OH-Δ^8-THC

Enzyme	Treatment	K_m (μM)	V_{max} (nmoles/min/mg protein)
Aniline hydroxylase	Control	65 ± 6	1.234 ± 0.033
	Δ^8-THC	67 ± 5	1.322 ± 0.030
	11-OH-Δ^8-THC	72 ± 4	1.483 ± 0.078*
p-Nitroanisole *O*-demethylase	Control	398 ± 27	1.309 ± 0.030
	Δ^8-THC	270 ± 39*	1.301 ± 0.056
	11-OH-Δ^8-THC	371 ± 24	1.596 ± 0.097*

Data represent the mean ±S.E. of four experiments. Δ^8-THC and 11-OH-Δ^8-THC were administered at a dose of 5 mg/kg, i.v., for 7 days. The concentrations of aniline and *p*-nitroanisole used as substrate were 0.1 to 2 mM.
* Significantly different from the control ($P < 0.05$).

to the incubation mixture consisting of potassium phosphate buffer, pH 7.4 (100 mM), $MgCl_2$ (10 mM), nicotinamide (4 mM), NADP (0.5 mM), glucose-6-phosphate (10 mM), glucose-6-phosphate dehydrogenase (4 units) and 0.7 ml of microsomal suspension in a final volume of 5 ml. The reaction mixture was incubated at 37° for 20 min and then extracted three times with 20 ml of ethylacetate. The combined extract was evaporated to dryness under N_2. The metabolic rate of Δ^8-THC or 11-OH-Δ^8-THC was determined by measuring the disappearance of the substrate in the extract, dissolving the residue obtained above in 0.5 ml of acetonitrile, and submitting it to gas chromatography (GC). GC conditions:a Shimadzu GC-6A gas chromatograph equipped with a flame ionization detector; column temp., 250° (Δ^8-THC) or 270° (11-OH-Δ^8-THC); detector temp., 280°; carrier gas, N_2, 50 ml/min; and injection volume, 2 μl.

The formation of 7α-OH-Δ^8-THC, 8α,9α-EHHC and 11-OH-Δ^8-THC was determined as follows: the residue obtained above was chromatographed on a Florisil column (1 g) and successively eluted with 5 ml each of *n*-hexane, benzene and ethylether. Δ^8-THC eluted in the benzene fraction, and other metabolites eluted in the ethylether fraction. After evaporation of the solvent under N_2, the metabolites were trimethylsilylated by adding 20 μl of acetonitrile, 10 μl of BSTFA, 5 μl of TMSI, and 5 μl of TMCS, and heating at 60° for 10 min. The trimethylsilylated metabolites were submitted to GC under the same conditions for the determination of Δ^8-THC. The recoveries of 7α-OH-Δ^8-THC, 8α,9α-EHHC and 11-OH-Δ^8-THC by this method were 79.1, 81.3 and 81.1% respectively.

RESULTS

The effects of repeated administration of Δ^8-THC and 11-OH-Δ^8-THC on relative liver weight are shown in Table 1. A significant decrease was observed in the Δ^8-THC-treated group on day 1 ($P < 0.05$) and in the 11-OH-Δ^8-THC-treated group on day 1 ($P < 0.01$) and day 7 ($P < 0.05$), whereas the relative liver weight of the control group remained constant.

Figure 1 shows the effects of the repeated administration of Δ^8-THC and 11-OH-Δ^8-THC on the hepatic microsomal drug-metabolizing enzyme system. On day 1, 11-OH-Δ^8-THC increased significantly aniline hydroxylase activity by 30%. On days 3 and 7, 11-OH-Δ^8-THC increased significantly the activities of both aniline hydroxylase by 21% and 18% and *p*-nitroanisole *O*-demethylase by 42% and 58%. Also increased significantly in microsomes from 11-OH-Δ^8-THC-treated mice was cytochrome P-450 content (by 34%) on day 3 and NADPH–cytochrome *c* reductase activity (by 35%) on day 7. In the Δ^8-THC-treated group, only aniline hydroxylase was increased significantly by 31% on day 7.

To determine the effects of Δ^8-THC and 11-OH-Δ^8-THC on kinetic parameters of aniline hydroxylase and *p*-nitroanisole *O*-demethylase, changes in K_m and V_{max} of both enzymes were studied in microsomes from mice treated with one or the other cannabinoids for 7 days (5 mg/kg/day, i.v.). Table 2

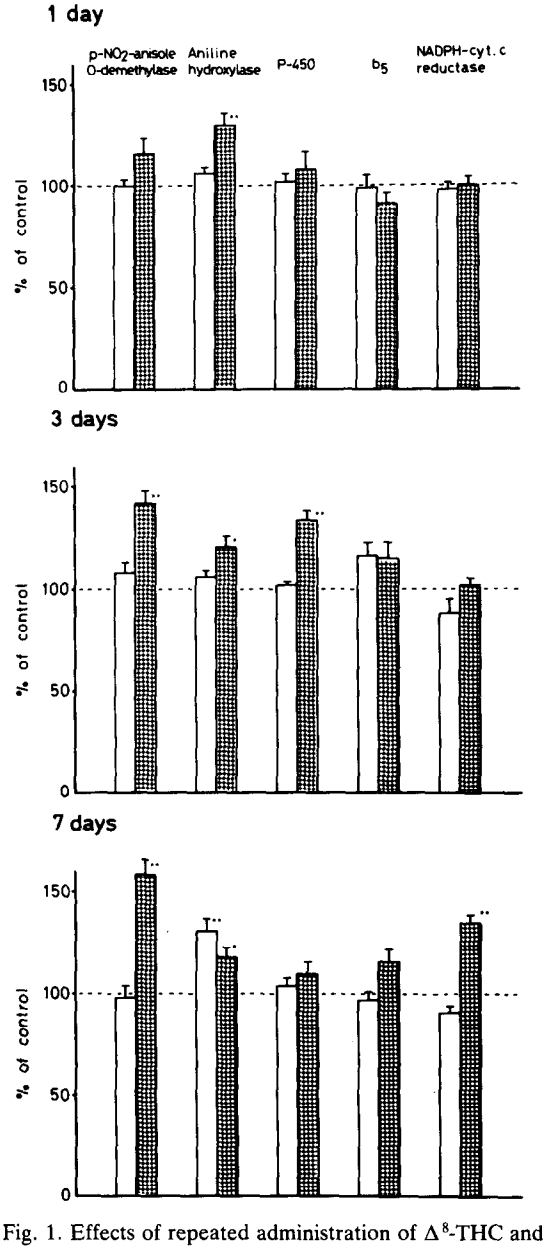

Fig. 1. Effects of repeated administration of Δ^8-THC and 11-OH-Δ^8-THC on the hepatic microsomal drug-metabolizing enzyme system of mice. Key: (□) Δ^8-THC-treated, and (▦) 11-OH-Δ^8-THC-treated. Values represent the mean (expressed as percent of control) ±S.E. of five or six experiments from ten or twelve mice. The mean values of enzyme activities and contents of controls are as follows: aniline hydroxylase, 0.973 to 1.075 nmoles *p*-aminophenol formed/min/mg protein; *p*-nitroanisole *O*-demethylase, 0.948 to 1.111 nmoles *p*-nitrophenol formed/min/mg protein; cytochrome P-450 content, 0.618 to 0.727 nmole/mg protein; cytochrome b_5 content, 0.376 to 0.469 nmole/mg protein; and NADPH–cytochrome *c* reductase, 121 to 125 nmoles/min/mg protein. Statistically significant differences are indicated by asterisks: (*) $P < 0.05$ and (**) $P < 0.01$.

indicates that 11-OH-Δ^8-THC-treatment increased significantly the V_{max} of both enzymes without affecting the K_m value, whereas Δ^8-THC-treatment did not affect the V_{max} of either enzyme but decreased

Table 3. Effects of repeated administration of Δ^8-THC or 11-OH-Δ^8-THC on their own metabolism with hepatic microsomes of mice

Treatment	Δ^8-THC metabolism	Metabolites formed from Δ^8-THC			11-OH-Δ^8-THC metabolism
		7α-OH-Δ^8-THC	8α,9α-EHHC	11-OH-Δ^8-THC	
Control (6)*	3.42 ± 0.17	0.72 ± 0.03	0.58 ± 0.07	1.48 ± 0.11	2.09 ± 0.06
Δ^8-THC-treated (5)	4.22 ± 0.12†	0.59 ± 0.03‡	0.76 ± 0.08	2.04 ± 0.22†	2.28 ± 0.09
11-OH-Δ^8-THC-treated (5)	4.45 ± 0.25†	0.73 ± 0.07	0.72 ± 0.08	2.23 ± 0.17†	2.62 ± 0.10‡

Data represent the mean (nmoles/min/mg protein) ±S.E.
* Number of experiments.
† Significantly different from the control ($P < 0.01$).
‡ Significantly different from the control ($P < 0.05$).

significantly ($P < 0.05$) the K_m value for *p*-nitroanisole *O*-demethylase (270 μM) as compared with the control (398 μM).

The effects of the repeated administration of Δ^8-THC or 11-OH-Δ^8-THC on their own metabolism with hepatic microsomes are shown in Table 3. The major metabolites of Δ^8-THC with hepatic microsomes of mice were 11-OH-Δ^8-THC, 7α-OH-Δ^8-THC and 8α,9α-EHHC. The sum of these metabolites formed accounted for about 80% of Δ^8-THC disappearance during incubation with all microsomes from control, Δ^8-THC- and 11-OH-Δ^8-THC-treated mice. The repeated administration of either cannabinoid led to an increase in the metabolism of Δ^8-THC with hepatic microsomes, which was attributable to an enhanced formation of 11-OH-Δ^8-THC, the most predominant metabolite of Δ^8-THC. The formation of 8α,9α-EHHC was not affected by either cannabinoid, but the formation of 7α-OH-Δ^8-THC was decreased significantly in hepatic microsomes from mice treated with Δ^8-THC. 11-OH-Δ^8-THC treatment also increased its own metabolism, although the effect of Δ^8-THC treatment was not significant in the metabolism of 11-OH-Δ^8-THC.

DISCUSSION

Marihuana extract and THC are known to have biphasic effects on the hepatic microsomal drug-metabolizing enzymes. An acute inhibitory effect, *in vitro* and *in vivo*, has been reported in experimental animals [27–32]. However, relatively limited information is available concerning the stimulative effect of cannabinoids on the hepatic microsomal drug-metabolizing enzymes [6, 7]. Lemberger *et al.* [3] reported that the plasma half-life of Δ^9-THC was significantly shorter in chronic users than in non-marihuana subjects. This supports the hypothesis that the repeated consumption of marihuana leads to a more rapid metabolism of Δ^9-THC. The present study demonstrates that repeated administration of Δ^8-THC or 11-OH-Δ^8-THC stimulates the hepatic microsomal drug-metabolizing enzymes of mice. 11-OH-Δ^8-THC, which is a major and active metabolite of Δ^8-THC in mice, produced a greater effect than did Δ^8-THC. Aniline hydroxylase and *p*-nitroanisole *O*-demethylase activities were increased significantly on and after the third administration of 11-OH-Δ^8-THC (5 mg/kg/day, i.v.). An increase in the activities was accompanied by an increase in cytochrome P-450 content (day 3) or NADPH–cytochrome *c* reductase (day 7). The stimulative effect of 11-OH-Δ^8-THC, therefore, may explain a quantitative change in the components of the microsomal electron transport system. Kinetic analysis indicated that the K_m values for both enzymes were not affected but the V_{max} values were increased, supporting the above view that a qualitative change in cytochrome P-450 species is not involved. In contrast, repeated administration of Δ^8-THC might cause a qualitative change in the components of the microsomal electron transport system. In Δ^8-THC-treated mice, the microsomal contents of cytochrome P-450 and b_5 as well as NADPH–cytochrome *c* reductase activity were not affected, and the V_{max} values of aniline hydroxylase and *p*-nitroanisole *O*-demethyl-

ase were almost the same as those in the control microsomes. On the other hand, the K_m value for *p*-nitroanisole *O*-demethylase was decreased significantly by Δ^8-THC-treatment. Δ^8-THC and Δ^9-THC are known to have high affinities to lipid rich membranes and to affect microsomal lipid components or conformation of lipid environment in microsomes [33]. These interactions may result in a change of kinetic characteristics of the microsomal drug-metabolizing enzymes. The specific nature of the change in the metabolism of Δ^8-THC was also observed in Δ^8-THC-treated microsomes. The formation of 7α-OH-Δ^8-THC was decreased significantly while the metabolism of Δ^8-THC and the formation of 11-OH-Δ^8-THC were increased significantly in microsomes from mice treated with Δ^8-THC. In 11-OH-Δ^8-THC treatment, the metabolism of Δ^8-THC and the formation of 11-OH-Δ^8-THC were increased significantly without affecting the formation of 7α-OH-Δ^8-THC. These results suggest that the repeated administration of Δ^8-THC may result in a change in cytochrome P-450 species or in microsomal lipid components, leading to a modification of catalytic activity of the microsomal drug-metabolizing enzymes. Whether Δ^8-THC treatment affects the actual formation of 7α-OH-Δ^8-THC or the further metabolism of this metabolite is not clear at present.

In conclusion, the repeated administration of 11-OH-Δ^8-THC as well as Δ^8-THC could affect microsomal metabolism of Δ^8-THC. Further studies, however, are necessary to understand more clearly enzyme induction with the cannabinoids, since the liver weight of mice was conversely decreased by the treatments with Δ^8-THC and 11-OH-Δ^8-THC. Whether the increase in their own metabolism by treatment with Δ^8-THC and 11-OH-Δ^8-THC may relate to their tolerance development also remains to be elucidated, while the increase in the formation of 11-OH-Δ^8-THC, which has greater potency in pharmacological effects and in tolerance development [16–19], is particularly worth noting.

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