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MICROSOMAL OXYGENASE CATALYZED OXIDATION OF 11-HYDROXY-Δ^8-TETRAHYDROCANNABINOL TO 11-OXO-Δ^8-TETRAHYDROCANNABINOL

Kazuhito Watanabe[1], Ikuo Yamamoto[1], Kazuta Oguri[2] and Hidetoshi Yoshimura[2*]

[1] School of Pharmacy, Hokuriku University, Kanazawa 920-11, Japan
[2] Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka 812, Japan

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SUMMARY: Rabbit liver microsomes were found to catalyze oxidation of 11-hydroxy-Δ^8-tetrahydrocannabinol to 11-oxo-Δ^8-tetrahydrocannabinol. This enzyme reaction required NADPH and molecular oxygen, and it was partially inhibited by CO. Pyrazole, potassium cyanide and sodium azide showed no effect on this oxidation, but SKF-525 A caused a significant inhibition. Thus, it is concluded that this enzymatic reaction is mediated by a mixed function oxidase involving cytochrome P-450.

It is well known that Δ^9-tetrahydrocannabinol (Δ^9-THC), an active component of marihuana, is metabolized to 11-hydroxy-Δ^9-THC (11-OH-Δ^9-THC) and further to Δ^9-THC-11-oic acid (1,2). Possible precursor of Δ^9-THC-11-oic acid was thought to be 11-oxo-Δ^9-THC, which was established recently as metabolite of Δ^9-THC with rat liver microsomes (3). Generally, oxidation of alcohol to aldehyde is catalyzed by dehydrogenases such as alcohol dehydrogenase. In spite of that, few examples were reported that oxygenase catalyzed an oxidation of primary or secondary alcohol to aldehyde or ketone, respectively (4-6). Since 11-OH-Δ^9-THC has still high lipophilic property, it seems to interact with microsomal membrane. In fact, we recently reported that 11-OH-Δ^8-THC, a metabolite of Δ^8-THC interact with rabbit liver

* To whom correspondence should be addressed.

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microsomes (7). It is therefore conceivable that both 11-OH-Δ^9-THC and 11-OH-Δ^8-THC are oxidized to 11-oxo-Δ^9-THC and 11-oxo-Δ^8-THC, respectively, by microsomal mixed function oxidase. Present study was investigated in order to establish this possibility with rabbit liver microsomes using 11-OH-Δ^8-THC as the substrate. This metabolic pathway involving 11-hydroxylation seems very important, because it is suggested that the pharmacological effects of THCs arise after its metabolic conversion to 11-OH-THCs which are more active than the parent compounds (8,9).

MATERIALS AND METHODS

NADPH and dansylchloride were obtained from Sigma Chemical Co. 2-Diethylaminoethyl-2,2-diphenylvalerate (SKF-525 A) was kindly supplied by Smith & Kline French Lab. 11-OH-Δ^8-THC and 11-oxo-Δ^8-THC were prepared as described in the previous report (10). Adult, male albino rabbit (2.0-2.5 kg) were used in this study and they were fasted for 24 hr prior to sacrifice. Cobaltous chloride ($CoCl_2 \cdot 6H_2O$) was administered subcutaneously at a dose of 20 mg/kg, twice a day for 3 days. Microsomes were prepared as previously reported (7). Incubations were carried out with microsomes equivalent to 0.2 g of liver, 50 µg of 11-OH-Δ^8-THC in 10 µl of acetone, 0.5 mM NADPH, 10 mM $MgCl_2$, 4 mM nicotinamide and 100 mM potassium phosphate buffer, pH 7.4, to make a final volume of 1.0 ml for 10 min at 37°. The reaction was terminated by addition of 0.2 M KCl-0.2 N HCl and extracted twice with 5 ml of ethylacetate. The extract was dansylated by modified method of Just et al. (11). The dansylated product was submitted to thin-layer chromatography (TLC) with the solvent system of benzene-hexane-ethylacetate (20:10:10) and few drops of mercaptoethanol in comparison with the authentic dansylated 11-oxo-Δ^8-THC (Rf 0.37) in darkness. After spraying of isopropanol-triethanolamine (4:1), the area corresponding to the dansylated 11-oxo-Δ^8-THC was scraped off under an ultraviolet lamp and extracted with 4 ml of chloroform containing 0.1 % mercaptoethanol. Dansylated 11-oxo-Δ^8-THC in the extract was determined fluorophotometrically with excitation at 360 nm and emission at 515 nm by Hitachi MPF-4 fluorescence spectrophotometer. Recovery of 11-oxo-Δ^8-THC from the incubation medium was quite low (55-60 %), but rather constant. Microsomal protein and cytochrome P-450 were determined according to the methods of Lowry et al. (12) and of Omura and Sato (13), respectively.

RESULTS AND DISCUSSION

When incubated with liver microsomes of rabbit as described above, 11-OH-Δ^8-THC was found to be oxidized to 11-

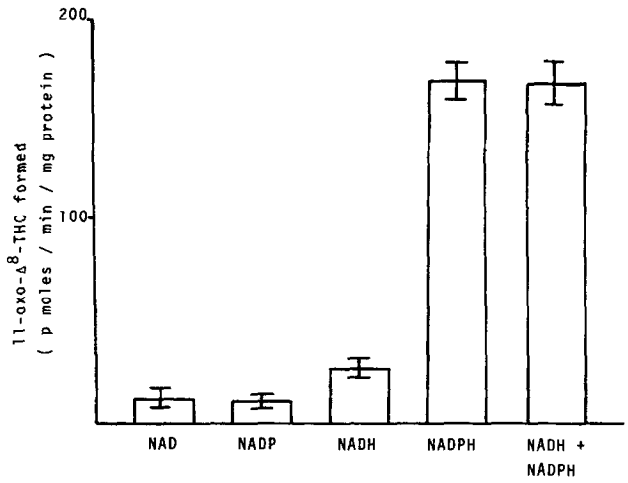

Fig. 1 Cofactor requirement on 11-oxo-Δ^8-THC formation with rabbit liver microsomes. Each cofactor was used at concentration of 0.5 mM. Figures represent the mean ± S.E. of 4 determinations.

oxo-Δ^8-THC by TLC examination. Maximum rate of this reaction was observed at pH range from 7.4 to 8.0 of the incubation medium. In addition, this enzyme reaction required reduced forms of pyridine nucleotides, of which NADPH was much more potent than NADH as a cofactor (Fig. 1). Synergism of two cofactors was not seen in this experimental condition.

As shown in Table I, the reaction proceeded only slightly under nitrogen atmosphere, indicating the requirement of oxygen. This low activity was very likely due to a small amount of remaining oxygen. Furthermore, replacement of air with mixed gas phase of CO and oxygen (4:1) caused loss of the activity (58 %). The effects of various enzyme inhibitors are also indicated in Table I. Although microsomal preparation contained residual alcohol dehydrogenase and catalase, addition of 1 mM pyrazole, a potent alcohol dehydrogenase inhibitor (14), to the incubation medium did not change the activity. In addition, neither 1 mM KCN nor NaN_3, both of which are the strong inhibitors of catalase (15,16), de-

Table I. Effects of inhibitors on 11-oxo-Δ^8-THC formation with rabbit liver microsomes.

	Concentration (mM)	Relative activity[a] to control (%)
pyrazole	1	102
KCN	1	109
NaN_3	1	86
SKF-525 A	1	35
carbon monoxide	$[CO]/[O_2]=4$ in the gas phase	42
nitrogen	100 % in the gas phase	14

a Reaction medium was the same as described in Materials and Methods except for CO and N_2 gas phase experiments, scales of which were 5 times as large as that of Materials and Methods.

Table II. Effect of pretreatment with $CoCl_2$ on 11-oxo-Δ^8-THC formation with rabbit liver microsomes.

	Control	$CoCl_2$
11-oxo-Δ^8-THC formed[a]	152 ± 12	111 ± 6*
Cytochrome P-450[b]	0.81 ± 0.03	0.47 ± 0.01**

a p moles 11-oxo-Δ^8-THC formed / min / mg protein
b n moles cytochrome P-450 / mg protein
* Significantly different from the control ($p<0.05$)
** Significantly different from the control ($p<0.01$)
Results represent the mean ± S.E. of 3 animals.

creased the activity. On the other hand, Table I also demonstrates the significant inhibition by SKF-525 A. These findings strongly suggested that this microsomal oxidation was mediated by neither alcohol dehydrogenase nor catalase, but by a mixed function oxidase involving cytochrome P-450. The mode of reaction was resemble to that of so-called microsomal ethanol oxidizing system (MEOS) but different in respect of inhibition with SKF-525 A (5,17). Involvement of

cytochrome P-450 in this microsomal oxidation was further supported by the pretreatment study with $CoCl_2$, a known inhibitor of cytochrome P-450 synthesis (18,19). Namely, the formation of 11-oxo-Δ^8-THC was significantly reduced with a concomitant decrease of cytochrome P-450 content, as summarized in Table II.

REFERENCES

1. Burstein, S., Rosenfeld, J., and Wittstruck, T. (1972) Science 176, 422-423.
2. Nordqvist, M., Agurell, S., Binder, M., and Nilsson, I. M. (1974) J. Pharm. Pharmacol. 26, 471-473.
3. Ben-Zvi, Z., and Burstein, S. (1974) Res. Comm. Chem. Path. Pharmacol. 8, 223-229.
4. Miller, M. L., Kalafer, M. E., Gayler, J. L., and Delwiche, C. V. (1967) Biochemistry 6, 2673-2678.
5. Lieber, C. S., and Decarli, L. M. (1968) Science 162, 917-918.
6. Nakamura, Y., Ueda, S., Iwatani, K., and Nakagawa, U. (1976) Eighth Synposium on Drug Metabolism and Action (Hiroshima, Japan) suppl, pp. 119-122.
7. Watanabe, K., Oguri, K., and Yoshimura, H. (1979) Life Sci. submitted.
8. Rosenfeld, R., Boegli, G., Testino, L., Brine, D. R., Pitt, C. G., and Wall, M. E. (1971) Science 172, 165-167.
9. Gill, E. W., and Jones, G. (1972) Biochem. Pharmacol. 21, 2237-2248.
10. Watanabe, K., Yamamoto, I., Oguri, K., and Yoshimura, H. (1979) Eur. J. Pharmacol. submitted.
11. Just, W. W., Erdmann, G., Werner, G., Wiechmann, M., and Thel, S. (1976) Marihuana pp. 123-138, Springer, New York.
12. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
13. Omura, T., and Sato, R. (1964) J. Biol. Chem. 239, 2370-2378.
14. Lundquist, F., Svendsen, L., and Petersen, P. H. (1963) Biochem. J. 86, 119-125.
15. Nicholls, P. (1964) Biochem. J. 90, 331-343.
16. Theorell, H., and Yonetani, T. (1963) Biochem. Z. 338, 537-553.
17. Lieber, C. S., and Decarli, L. M. (1970) J. Biol. Chem. 245, 2505-2512.
18. Tephly, T. R., and Hibbeln, P. (1971) Biochem. Biophys. Res. Commun. 42, 589-595.
19. Guzelian, P. S., and Bissell, D. M. (1976) J. Biol. Chem. 251, 4421-4427.