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Cannabielsoin as a New Metabolite of Cannabidiol in Mammals

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YAMAMOTO, I., H. GOHDA, S. NARIMATSU, K. WATANABE AND H. YOSHIMURA. *Cannabielsoin as a new metabolite of cannabidiol in mammals.* PHARMACOL BIOCHEM BEHAV **40**(3) 541–546, 1991.—Cannabielsoin (CBE) was identified as a novel metabolite of cannabidiol (CBD) in the guinea pig in vivo and in vitro. Its formation by liver microsomes of guinea pigs needed NADPH and molecular oxygen, and was inhibited with SKF 525-A, metyrapone and α-naphthoflavone, indicating participation of cytochrome P-450 (P-450). The CBE-forming activity was highest in guinea pigs, followed by mice, rabbits and rats. In the rat, sex difference was found in the CBE formation (male>female). CBD monomethylether (CBDM) was also biotransformed to CBE monomethylether (CBEM) in the guinea pig in vivo and in vitro. When CBD dimethylether (CBDD) was employed as substrate, 1S,2R-epoxy-CBDD was identified. The results suggest that CBD and CBDM are biotransformed by P-450 to CBE-type metabolites via 1S,2R-epoxides. In pharmacological studies using mice, CBDD and 1S, 2R-epoxy-CBD-2′,6′-diacetate produced hypothermia, and CBD, CBDM and CBEM prolonged pentobarbital-induced sleep. Moreover, 1S,2R-epoxy-CBD-2′,6′-diacetate was examined in the Ames test, but had no mutagenicity.

Cannabidiol Cannabielsoin 1S,2R-Epoxy intermediate Guinea pig Liver microsomes Cytochrome P-450 Species difference Hypothermia Barbiturate synergism Ames test

CANNABIDIOL (CBD) is a promising anticonvulsant candidate (13). Metabolism of CBD has extensively been studied in various animal species (1, 6, 11, 12, 21). We have previously reported that epoxidation is one of the metabolically activating pathways of tetrahydrocannabinols (THCs), which are psychoactive components of marihuana (14,19). It is, therefore, important to study epoxide-forming pathways in the oxidative metabolism of CBD. Recently, Harvey and Mechoulam have identified dihydrodiol metabolite of CBD, which is thought to be formed from 8,9-epoxy-CBD, in human urine (7). However, there are no systematic reports focused on the biological formation and pharmacological effects of epoxy metabolites of CBD. We described here identification and determination in various animal species, especially in guinea pigs, of cannabielsoin (CBE) which is thought to be biologically formed from CBD via 1,2-epoxy intermediate, and its formation mechanism (22). Moreover, we assessed pharmacological and toxicological effects of CBD and its related cannabinoids using hypothermia, barbiturate synergism and Ames test as indices.

METHOD

Materials

NAD, NADP, NADH, NADPH and glucose-6-phosphate (G-6-P) were purchased from Boehringer-Mannheim GmbH (Darmstadt, FRG); G-6-P dehydrogenase (type V, EC 1.1.1.49), Fast Blue BB salt, metyrapone and α-naphthoflavone were from Sigma Chemical Co. (St. Louis, MO); TMS derivatizing reagents [N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilylimidazole and trimethylchlorosilane] were from Tokyo Kasei Co. (Tokyo, Japan); SKF 525-A was from Smith, Kline & French Lab. (Philadelphia, PA); Sephadex LH-20 was from Pharmacia Fine Chemicals (Uppsala, Sweden). CBD was isolated and purified from cannabis leaves by the method previously reported (2). CBE, 1S,2R-epoxy-CBD-2′,6′-diacetate, 1R,2S-epoxy-CBD, CBD monomethylether (CBDM), CBD dimethylether (CBDD), and 1S,2R-epoxy-CBDD were prepared as reported previously (5). Purities of the cannabinoids were checked by GC to be at least above 95%. Other chemicals used were of the best quality commercially available.

Animals

Adult Hartley guinea pigs (350–410 g), Sprague-Dawley rats (165–220 g), ddN mice (21–25 g) and Japanese white rabbits (2.5–3.1 kg) were obtained from Hokuriku Experimental Animals Lab. (Kanazawa, Japan). The animals were kept in air-conditioned rooms with 12-h light cycle (0700–1900), and given food and water ad lib.

In Vitro Metabolism of Cannabinoids

Animals were killed after starvation for 24 h, and livers were perfused with 0.9% NaCl. Microsomes suspension was prepared

by the reported method (16). Typical reaction medium consisted of NADP (5 μmol), G-6-P (50 μmol), $MgCl_2$ (90 μmol), G-6-P dehydrogenase (5 units), and potassium phosphate buffer (100 mM, pH 7.4) to make a final volume of 9.0 ml. Reaction at 37°C was started by adding cannabinoids (500 μg) and microsomes (1 ml, equivalent to 1 g liver). After incubation for appropriate time, the reaction was stopped and metabolites were extracted by adding ethylacetate (25 × 2 ml) and shaking. The extract after evaporation of the solvent was subjected to GC and GC/MS after TMS derivatization, if necessary. Metabolites were identified by comparison with synthetic standards, and tentatively identified by referring published fragmentations (1, 7, 12, 13) when synthetic standards were not available. Approximate ratios of metabolite formation were calculated from peak areas on total ion chromatograms in GC/MS. When metabolites were determined by GC or HPLC, the volume of reaction medium was made to one-fifth or one-tenth that for metabolite identification described above. Cannabinoids (CBE, CBEM, 1S,2R- and 1R,2S-epoxy-CBDD) were determined on the basis of calibration curves made by adding known amounts of the synthetic standards to the incubation medium containing boiled microsomes instead of fresh ones.

In Vivo Metabolism of Cannabinoids

Guinea pigs were given CBD, CBDM or CBDD (suspended in 0.9% NaCl containing 1% Tween 80, 100 mg/kg, IP), and killed 1 h later. Metabolites in the liver were extracted with acetone, and fractionated with Sephadex LH-20 (1 × 15 cm) using chloroform containing 2 to 16% of methanol as a solvent system according to the method reported by Harvey et al. (6). Metabolites were then analyzed by GC and GC/MS after TMS derivatization, if necessary.

GC and GC/MS GC

A Shimadzu GC/5A gas chromatograph equipped with a hydrogen flame ionization detector and a glass column packed with 2% OV-17 or 3% SE-30 on Chromosorb W (60–80 mesh, 3 mm × 1.5 m) were used. Column temperature, 250°C; detector temperature, 275°C; carrier gas, N_2 50 ml/min. GC/MS: a JEOL JMS-300 mass spectrometer equipped with a JEOL GCG-06 gas chromatograph and a JEOL JMA DA-5000 mass data system. Column; a glass column packed with 2% OV-17 or 3% SE-30 on Chromosorb W (60–80 mesh, 3 mm × 1.5 m); column temperature, 250°C; injection port temperature, 275°C; carrier gas, He 40 ml/min; ionization current, 0.3 mA; ionization energy, 70 eV.

HPLC

A Hitachi 655 type liquid chromatograph equipped with a 655 type variable wave length UV monitor, 655-60 type data processor, and a DuPont Zorbax ODS column (4.6 mm × 15 cm) were used. A mobile phase, methanol/acetonitrile/water (80:10:10, v/v/v); flow rate, 1 ml/min; detection wave length, 280 nm.

Pharmacological Effects of Cannabinoids

Cannabinoids to be examined were suspended in 0.9% NaCl containing 1% Tween 80, and injected into mice through a tail vein (0.1 ml/10 g of body weight). Pharmacological activities were measured using indices of hypothermia and barbiturate synergism as previously reported (20). Mutagenicity of 1S,2R-epoxy-CBD-2′,6′-diacetate dissolved in dimethylsulfoxide was assessed by the reported method (3) using *Salmonella typhimurium* TA98 and TA100 as tester strain and benz(a)-pyrene and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) as positive control.

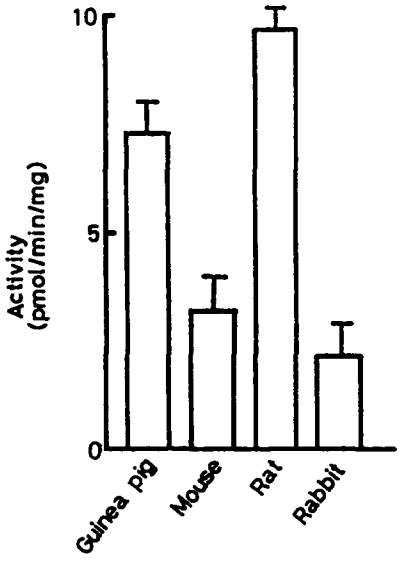

FIG. 1. Species difference in CBEM formation from CBDM with liver microsomes. Each bar represents the mean value ± S.E. of 3 determinations.

Enzyme Assay

Aniline hydroxylase and p-nitroanisole O-demethylase activities were determined by the methods of Imai et al. (9) and of Kato and Gillette (10), respectively. Cytochrome P-450 contents were measured by the method of Omura and Sato (17). Protein concentration was determined by the Lowry method (11).

Statistics

Statistical significance was calculated by the Student's *t*-test.

RESULTS AND DISCUSSION

Characterization of CBE Formation From CBD (22)

CBD was incubated with liver microsomes of the guinea pig in the presence of an NADPH-generating system, and metabolites extracted with EtOAc were converted into trimethylsilyl derivatives. 2TMS-CBE [retention time 3.4 min on 3% SE-30 column, characteristic fragment ions at m/z 474 (M^+, 12% as relative intensity), 391 (13%), 130 (79%), 108 (100%)] was identified as one of oxidation metabolites of CBD by GC/MS. On the basis of peak areas of total ion and mass chromatograms in GC/MS, the most abundant metabolite was 7-hydroxy-CBD (7-OH-CBD, 100% as relative abundance) followed by 6-OH-CBD (a mixture of 6α- and 6β-OH-CBD, 52%), 4″-OH-CBD (34%), CBE (16%) and 2″-OH-CBD (15%). The CBE-forming activity was highest in the guinea pig (18.3 pmol/min/mg of protein) among 4 animal species examined (mouse, 3.6; rabbit, 3.0; rat, 1.9 pmol/min/mg of protein). Moreover, the activity towards the CBE formation was higher in male rats than in female ones, while none of sex difference was observed in other

TABLE 1

SEX DIFFERENCE IN CBE FORMATION AND DRUG-METABOLIZING ENZYME ACTIVITIES IN LIVER MICROSOMES OF GUINEA PIGS, MICE AND RATS

Species	Sex	Cyt. P-450 Contents (nmol/mg)	CBE Formation (pmol/min/mg)	AN Hydroxylase (nmol/min/mg)	p-NA O-Demethylase (nmol/min/mg)
Guinea pig	Male	1.20 ± 0.10	18.0 ± 4.3	0.43 ± 0.04	2.44 ± 0.08
	Female	1.44 ± 0.18	21.7 ± 4.0	0.53 ± 0.06	3.45 ± 0.28
Mouse	Male	1.09 ± 0.05	3.5 ± 0.2	0.48 ± 0.06	1.22 ± 0.14
	Female	0.97 ± 0.20	2.6 ± 0.1	0.63 ± 0.05	1.34 ± 0.16
Rat	Male	1.02 ± 0.08	1.9 ± 0.2	1.30 ± 0.15	1.34 ± 0.24
	Female	0.77 ± 0.11	0.8 ± 0.1*	0.51 ± 0.12†	1.01 ± 0.05

Each value represents the mean ± S.E. of 4 determinations. AN: aniline; p-NA: p-nitroanisole. *Significantly different from male ($p<0.05$). †Significantly different from male ($p<0.01$).

animal species such as guinea pigs or mice (Table 1).

For the CBE formation with guinea pig liver microsomes, NADPH as a cofactor and molecular oxygen were necessary. SKF 525-A, metyrapone and α-naphthoflavone (0.5 mM each) significantly inhibited the CBE-forming activity by 67, 54 and 71%, respectively. Effects of pretreatment of guinea pigs with phenobarbital or 3-methylcholanthrene on the CBE formation are summarized in Table 2. The treatments caused significant increase in cytochrome P-450 contents and p-nitroanisole O-demethylation, but the activity towards CBE formation was not influenced neither by phenobarbital nor 3-methylcholanthrene-treatment.

The results from in vitro experiments using various cytochrome P-450 inhibitors indicate that the CBE formation is mediated by monooxygenase system including cytochrome P-450. On the other hand, in vivo experiments using enzyme inducers did not positively support the participation of the monooxygenase system in the CBE formation. We think there are two possibilities for the explanation of these results. One of them is that CBE formation may be mediated by cytochrome P-450 isozyme which is different from those induced by phenobarbital and 3-methylcholanthrene. The other is that enzyme induction by the inducers may contribute not only to the CBE formation from CBD but also to further oxidative metabolism of CBE, resulting in the unaltered level of CBE before and after the inducer treatments.

TABLE 2

CBE FORMATION AND DRUG-METABOLIZING ENZYME ACTIVITIES IN LIVER MICROSOMES OF GUINEA PIGS TREATED WITH INDUCERS

Treatment	Cyt. P-450 Contents (nmol/mg)	CBE Formation (pmol/min/mg)	p-NA O-Demethylase (nmol/min/mg)
Control	1.03 ± 0.08	18.2 ± 1.2	0.44 ± 0.07
PhB	1.44 ± 0.16*	16.9 ± 1.4	1.68 ± 0.27*
3-MC	1.81 ± 0.35*	19.9 ± 2.3	5.42 ± 1.86*

Guinea pigs were given phenobarbital (PhB, 40 mg/kg, IP, 3 times) or 3-methylcholanthrene (3-MC, 40 mg/kg, IP, once), and killed 24 h after the last injection. Liver microsomes were prepared, and activities were measured as described in the Method section. p-NA: p-nitroanisole. Each value represents the mean ± S.E. of 4 determinations. *Significantly different from the control ($p<0.05$).

One hour after pretreatment of the guinea pig with CBD (100 mg/kg, IP), the liver was excised and metabolites were extracted by acetone. Among metabolites, CBE was identified as one of in vivo metabolites by GC/MS analysis (data not shown).

Characterization of CBEM Formation From CBDM (5)

When CBDM was incubated with liver microsomes of the guinea pig fortified with an NADPH-generating system, CBEM [as a TMS derivative, retention time, 3.4 min on 2% OV-17 column, characteristic fragment ions at m/z 416 (M^+, 9%), 333 (11%), 130 (83%), 108 (100%)] was formed together with several monohydroxylated and dihydroxylated metabolites. Identified metabolites were 7-OH-CBDM (100% as relative abundance), 6-OH-CBDM (a mixture of 6α- and 6β-OH-CBDM, 96%), CBEM (7%), 6,7-diOH-CBDM (2%) and 7,2''-diOH-CBDM (2%). None of demethylation of CBDM and CBEM was observed under the conditions used here.

The microsomal CBEM formation from CBDM also needed NADPH as a cofactor and molecular oxygen, and was significantly inhibited by SKF 525-A (0.5 mM), suggesting that as with the CBE formation from CBD, this reaction may also be

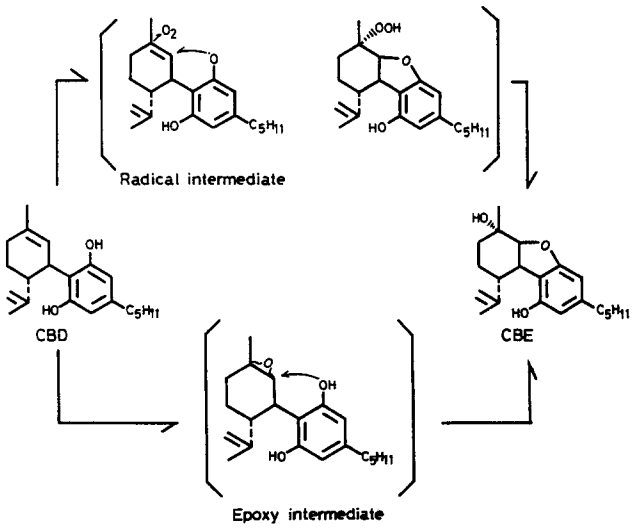

FIG. 2. Possible pathways of biological CBE formation from CBD.

TABLE 3

IDENTIFICATION OF 1S,2R-EPOXY-CBDD AS AN IN VITRO METABOLITE OF CBDD IN THE GUINEA PIG

	Retention Time (min)	Fragment Ions (m/z)
Biologic metabolite	6.9	358 (M^+,6), 290 (11), 250 (52), 221 (100), 208 (13), 152 (13)
Synthetic 1S,2R-epoxy-CBDD	6.9	358 (M^+,18), 290 (28), 250 (39), 221 (100), 208 (19), 152 (22)

CBDD metabolites were subjected to GC/MS using 2% OV-17 column (3 mm × 1.5 m). Column temperature, 250 °C; ionization energy, 70 eV; ionization current, 300 μA; carrier gas, He 40 ml/min. Numbers in parentheses are relative intensities (%).

mediated by the hepatic monooxygenase system. Figure 1 shows species difference in microsomal CBEM formation from CBDM. The rat exhibited the highest activity followed by the guinea pig, mouse and rabbit. Species difference between CBD and CBDM in formation of elsoin type metabolite suggests that species variation exists in a particular form of cytochrome P-450 to catalyze the formation of elsoin type metabolite toward each cannabinoid. As in the case of CBE formation, in vivo formation of CBEM from CBDM in the guinea pig was confirmed by GC/MS analysis (data not shown).

Identification of an Epoxy Metabolite Formed From CBDD (5)

As described above, we confirmed the microsomal formation of cannabielsoin-type metabolites from CBD and CBDM. Shani and Mechoulam (20) have found CBE as one of components of marihuana, and have proposed a mechanism involving a radical intermediate for CBE formation (Fig. 2, upper pathway).

On the other hand, Uliss et al. have reported that CBE was chemically produced from CBD via 1S,2R-epoxy-CBD as an intermediate (19). Taking account of the results obtained here, we

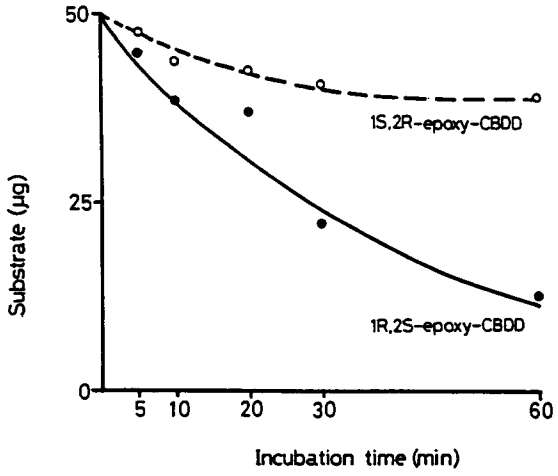

FIG. 3. Difference in metabolism of 1S,2R- and 1R,2S-epoxy-CBDD with liver microsomes of the guinea pig. Each epoxide (50 μg) was incubated with liver microsomes in the presence of an NADPH-generating system at 37°C for appropriate time. Remaining substrates were extracted with ethylacetate, and determined by HPLC under the conditions described in the Method section. Each point is mean value of two determinations.

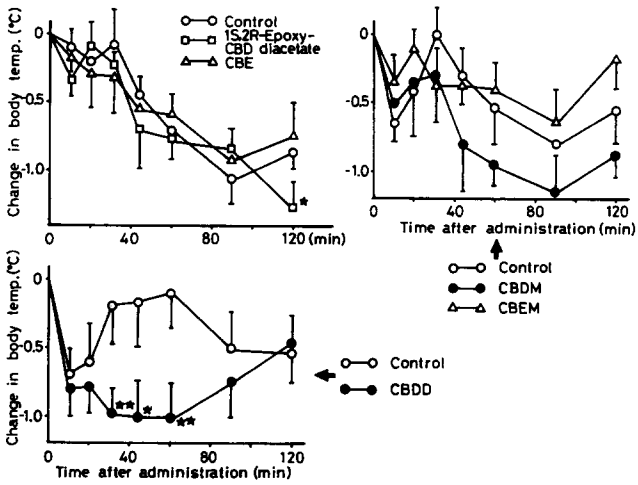

FIG. 4. Effect of CBE and related cannabinoids on body temperature of the mouse. Each cannabinoid (10 mg/kg, IV) was given to mice, and rectal temperature was measured by a thermistor probe. Control mice were given with only vehicle (1% Tween 80-saline). Points are mean values ± S.E. of 10 mice. *Significantly different from the control ($p<0.05$). **Significantly different from the control ($p<0.01$).

also think that CBD and CBDM are biotransformed to epoxy intermediates by the monooxygenase system, and then quickly converted into elsoin-type metabolites by attacking a phenoxy anion at the 2′-position on the carbon atom at the 2-posi-

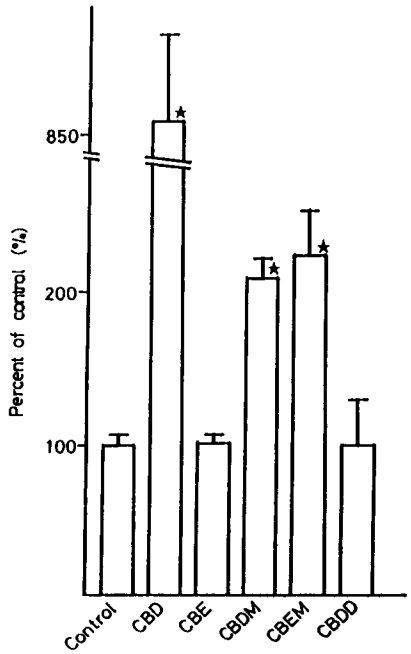

FIG. 5. Effect of CBE and related cannabinoids on pentobarbital-induced sleep. Mice were pretreated with cannabinoids (10 mg/kg, IV), and sodium pentobarbital (40 mg/kg, IP) was given 5 min later. Sleeping time was measured as the time during loss of righting reflex. Control mice were injected with vehicle (1% Tween 80-saline) instead of the cannabinoids. Each value is mean ± S.E. of 10 mice. The sleeping time of the control group was 23 ± 2 min. *Significantly different from the control ($p<0.05$).

TABLE 4

MUTAGENICITY TESTING 1S,2R-EPOXY-CBD-2′,6′-DIACETATE IN THE SALMONELLA/S9 MIX ASSAY

	Dose (μg per plate)							
	0	5	10	50	100	500	1000	Positive Control
TA98 (+S9)	32	18	24	16	14	19	14	218 [B(a)P]
TA98 (−S9)	8	17	17	16	12	16	19	138 [AF-2]
TA100 (+S9)	110	116	114	104	78	244	92	791 [B(a)P]
TA100 (−S9)	87	58	66	62	60	68	63	250 [AF-2]

Numbers are of His^+ revertants. As the positive control, benzo(a)-pyrene [B(a)P, 0.5 μg per plate] or 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2, 0.1 μg per plate) was added to the medium with or without S9 mix, respectively. Values were mean of two determinations.

tion as shown in Fig. 2 (lower pathway). If it is the case, identification of an epoxy intermediate is possible by using CBDD as a substrate, phenolic hydroxyl groups of which are masked by methyl groups. We then incubated CBDD with liver microsomes under similar conditions for CBE and CBEM identification described above. As shown in Table 3, 1S,2R-epoxy-CBDD was identified as one of microsomal oxidation metabolites of CBDD in the guinea pig. Moreover, we succeeded in identification of 1S,2R-epoxy-CBDD in the guinea pig as an in vivo metabolite of CBDD (data not shown).

Metabolism of Epoxides of CBDD

1S,2R- and 1R,2S-epoxy-CBDD were incubated with liver microsomes in the presence of an NADPH-generating system for an appropriate time, and remaining epoxides were determined by HPLC (Fig. 3). 1R,2S-epoxy-CBDD was metabolized more quickly than 1S,2R-epoxy-CBDD, and 68% of the 1R,2S-epoxide and 24% of 1S,2R-epoxide were metabolized after 60 min of incubation period. Biological half-lives were 30 and 75 min for 1R,2S- and 1S,2R-epoxy-CBDD, respectively. It is likely that considerable differences may exist in rates of further metabolism of the epoxides by liver microsomes of the guinea pig, i.e., in the oxidation by the mixed function oxidase system or in the hydrolysis by epoxide hydrolase.

The results obtained from the present metabolic studies indicate that CBD and CBDM are biotransformed to 1S,2R-epoxides by the hepatic microsomal monooxygenase system including cytochrome P-450, and the epoxides are immediately and may be spontaneously converted to elsoin-type metabolites.

Pharmacological Activities

We have examined pharmacological effects in mice of various metabolites of THCs using several indices such as catalepsy, hypothermia and barbiturate synergism, and indicated contribution of some active metabolites to pharmacological effects produced by the parent cannabinoids (21). We thus examined pharmacological activities in mice of the synthetic cannabinoids used in the present study. Figure 4 shows the effects of CBE and its related cannabinoids on the body temperature of mice. Among five cannabinoids tested, CBDD significantly lowered the body temperature 30 to 60 min after the injection. 1S,2R-epoxy-CBD-2′,6′-diacetate lowered the temperature only one point time interval at 120 min after the injection, but no other cannabinoids showed any significance in the change of body temperature.

Pentobarbital-induced sleep prolongation was assessed with five cannabinoids in mice (Fig. 5). CBD showed the highest activity (8.5-fold of the control sleeping time) followed by CBEM (2.1-fold) and CBDM (2.0-fold). CBE and CBDD did not show any effect in this index, suggesting that these cannabinoids were without the inhibitory effect on the liver metabolism of pentobarbital.

It is well known that some arene and alkene compounds are biotransformed to their epoxides, exhibiting their mutagenic and carcinogenic toxicities. Therefore, it is worthwhile to examine mutagenicity of epoxy metabolites of CBD and its related compounds. Since the epoxide was not available as itself, 1S,2R-epoxy-CBD-2′,6′-diacetate was chosen for the Ames test. Table 4 summarizes results of the test using *Salmonella typhimurium* TA98 and TA100. Comparing the results of benz(a)pyrene and AF-2 as the positive control, the epoxy derivative of CBD did not show mutagenic activity at any doses employed regardless of adding S9 mix. Glatt et al. have reported that Δ9-tetrahydrocannabinol and its epoxide (9,10-epoxyhexahydrocannabinol) did not have mutagenic activity in the Ames test (4).

The results from the pharmacological experiments indicate that among the cannabinoids tested, CBDM, CBEM, CBDD and 1S,2R-epoxy-CBD-2′,6′-diacetate have some activities in hypothermia or barbiturate synergism as index. We could not assess pharmacological activities of other cannabinoids such as 1S,2R- and 1R-2S-epoxy-CBDD in the present study. Furthermore, since elsoin-type metabolites used in the present study still have considerable lipophilicity as judged from their behaviour in TLC and GC, there is a possibility of discovering unique properties if we choose appropriate pharmacological index. We are now further studying on this line.

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REFERENCES

1. Agurell, S.; Halldin, M.; Lindgren, J.-E.; Ohlsson, A.; Widman, M.; Gillespie, H.; Hollister, L. Pharmacokinetic and metabolism of Δ^1-tetrahydrocannabinol and other cannabinoids with emphasis on man. Pharmacol. Rev. 38:21–44; 1986.
2. Aramaki, H.; Tomiyasu, N.; Yoshimura, H.; Tsukamoto, H. Forensic chemical study on marihuana. I. A detection method of the principal constituents by thin-layer and gas chromatographies. Chem. Pharm. Bull. 16:822–126; 1968.
3. Demura, R.; Tsukada, S.; Kotani, N.; Tateoka, Y.; Narimatsu, S.; Yamamoto, I. Mutagenic activity of pyrolysates of cyanocobalamin

and some other water-soluble vitamins in the model system with the Salmonella/mammalian microsomes. Mutat. Res. 244:37–42; 1990.
4. Glatt, H.; Ohlsson, A.; Agurell, S.; Oesch, F. Δ^1-Tetrahydrocannabinol and 1α, 2α-epoxyhexahydrocannabinol: Mutagenicity investigation in the Ames test. Mutat. Res. 66:329–335; 1979.
5. Gohda, H.; Narimatsu, S.; Yamamoto, I.; Yoshimura, H. In vivo and in vitro metabolism of cannabidiol monomethylether and cannabidiol dimethylether in the guinea pig: On the formation mechanism of cannabielsoin-type metabolite from cannabidiol. Chem. Pharm. Bull. 38:1697–1701; 1990.
6. Harvey, D. J.; Martin, B. R.; Paton, W. D. M. Identification and measurement of cannabinoids and their in vivo metabolites in liver by gas chromatography-mass spectrometry. In: Nahas, G. G.; Paton, W. D. M., eds. Marihuana: Biological effects. Advances in the biosciences, vols. 22 and 23. Oxford: Pergamon Press; 1979:45–62.
7. Harvey, D. J.; Martin, B. R.; Paton, W. D. M. Identification of in vivo liver metabolites of Δ^1-THC, CBD and CBN produced by the guinea pig. J. Pharm. Pharmacol. 32:267–271; 1980.
8. Harvey, D. J.; Mechoulam, R. Metabolites of cannabidiol identified in human urine. Xenobiotica 20:303–320; 1990.
9. Imai, Y.; Itoh, A.; Sato, R. Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. J. Biochem. (Tokyo) 60:417–428; 1966.
10. Kato, R.; Gillette, J. R. Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. J. Pharmacol. Exp. Ther. 150:279–284; 1965.
11. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
12. Martin, B.; Nordqvist, M.; Agurell, S.; Lindgren J.-E.; Leander, K.; Binder, M. Identification of monohydroxylated metabolites of cannabidiol formed by rat liver. J. Pharm. Pharmacol. 28:275–279; 1976.
13. Martin, B.; Harvey, D. J.; Paton, W. D. M. Biotransformation of cannabidiol in mice. Identification of new acid metabolites. Drug Metab. Dispos. 5:259–267; 1977.
14. Nahas, G. G. The medical use of cannabis. In: Nahas, G. G., ed. Marihuana in science and medicine. New York: Raven Press; 1984: 247–261.
15. Narimatsu, S.; Yamamoto, I.; Watanabe, K.; Yoshimura, H. 9α, 10α-Epoxyhexahydrocannabinol formation from Δ^9-tetrahydrocannabinol by liver microsomes of phenobarbital-treated mice and its pharmacological activities in mice. J. Pharmacobio-Dyn. 6:558–564; 1983.
16. Narimatsu, S.; Matsubara, K.; Shimonishi, T.; Watanabe, K.; Yamamoto, I.; Yoshimura, H. Enzymatic oxidation of 7-hydroxylated Δ^8-tetrahydrocannabinol to 7-oxo-Δ^8tetrahydrocannabinol by hepatic microsomes of the guinea pig. Drug Metab. Dispos. 16:156–161; 1988.
17. Omura, T.; Sato, R. The carbon-monoxide binding pigment of liver microsomes. II. Solubilization, purification and properties. J. Biol. Chem. 239:2370–2378; 1964.
18. Shani, A.; Mechoulam, R. Cannabielsoic acids: Isolation and synthesis by a novel oxidative cyclization. Tetrahedron 30:2437–2446; 1974.
19. Uliss, D. B.; Razdan, R. K.; Dalzell, H. C. Stereospecific intramolecular cleavage by phenolate anion; synthesis of novel and biologically active cannabinoids. J. Am. Chem. Soc. 96:7372–7374; 1974.
20. Yamamoto, I.; Narimatsu, S.; Watanabe, K.; Yoshimura, H. Pharmacological activity of 8α,9α- and 8β,9β-epoxyhexahydrocannabinol in mice. Res. Commun. Subst. Abuse 2:409–417; 1981.
21. Yamamoto, I. Metabolic activation of marihuana constituents: Relations to pharmacological and toxicological effects. Yakugaku Zasshi 106:537–561; 1986.
22. Yamamoto, I.; Gohda, H.; Narimatsu, S.; Yoshimura, H. Identification of cannabielsoin, a new metabolite of cannabidiol formed by guinea-pig hepatic microsomal enzymes, and its pharmacological activity in mice. J. Pharmacobio-Dyn. 11:833–838; 1988.