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Pharmacological Activities in the Mouse of Δ^9-Tetrahydrocannabinol Metabolites Oxidized at the 8-Position

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The pharmacological effects of Δ^9-tetrahydrocannabinol (THC) metabolites, 8α-hydroxy-Δ^9-THC (8α-OH-Δ^9-THC), 8β-OH-Δ^9-THC and 8-oxo-Δ^9-THC, were examined in the mouse. Cataleptogenic, hypothermic and pentobarbital-induced sleep-prolonging effects were produced by intravenous injection of the metabolites and compared with those of Δ^9-THC. In all the pharmacological indices, Δ^9-THC and 8-oxo-Δ^9-THC had the highest and the lowest activity, respectively. The activity of 8α-OH-Δ^9-THC was less potent in catalepsy and more potent in hypothermia than that of 8β-OH-Δ^9-THC, while they were equipotent in barbiturate synergism. These results suggest that oxidation at the 8-position is one of the detoxication pathways of Δ^9-THC, and also indicate the complexity of cannabinoid action in the central nervous system.

Keywords——Δ^9-tetrahydrocannabinol; 8α-hydroxy-Δ^9-tetrahydrocannabinol; 8β-hydroxy-Δ^9-tetrahydrocannabinol; 8-oxo-Δ^9-tetrahydrocannabinol; catalepsy; hypothermia; pentobarbital-induced sleep prolongation

The metabolism of Δ^9-tetrahydrocannabinol (THC), a major psychotropic constituent of marihuana,[1,2] has been extensively studied *in vitro* and *in vivo*.[3] The main metabolic pathways are oxidations at the allylic 8- and 11-carbons and on the pentyl side-chain.[4] Various lines of evidence indicate that oxidation at the 9-methyl group is a metabolically activating pathway (see a review by Lemberger.[3]). On the other hand, there are conflicting data on the pharmacological activities of Δ^9-THC metabolites oxidized at the 8-position. That is, Perez-Reyes *et al.*[5] and Hollister[6] have reported that the pharmacological activity in man of 8β-hydroxy-Δ^9-THC (8β-OH-Δ^9-THC) was more potent than that of 8α-OH-Δ^9-THC, whereas Wilson and May[7] and Consroe *et al.*[8] found higher activity of 8α-OH-Δ^9-THC than of 8β-OH-Δ^9-THC in mice and rabbits, respectively. Further, Ben-Zvi *et al.* have found no difference in activity between 8α-OH- and 8β-OH-Δ^9-THCs in rhesus monkeys.[9] These inconsistent results are probably due to the difference in the animal species and pharmacological indices used by the investigators. Furthermore, pharmacological effects of 8-oxo-Δ^9-THC, which is an oxidized metabolite of 8-OH-Δ^9-THCs, have not been reported, although Mechoulam *et al.*[10] observed no activity of 1-acetyl-8-oxo-Δ^9-THC in rhesus monkeys.

In the present study, the pharmacological activities of 8α-OH-, 8β-OH- and 8-oxo-Δ^9-THCs in the mouse were assessed on the basis of three indices and compared with those of the parent compound to evaluate the contribution of oxidative metabolism at the 8-position to the pharmacological effects of Δ^9-THC.

Experimental

Synthesis of Metabolites——Δ^9-THC was isolated from cannabis leaves and purified according to the reported method.[11] 8α-OH- and 8β-OH-Δ^9-THCs were synthesized from Δ^9-THC by the method of Pitt *et al.*[12] 1-Acetyl-8-oxo-Δ^9-THC (180 mg) prepared by the published method[12] was dissolved in 10 ml of ethanol containing 0.1 N NaOH, and the solution was stirred at room temperature for 2 h. After addition of H_2O (20 ml), the solution was shaken with diethyl ether. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under an N_2 stream. The residue was subjected to preparative thin-layer chromatography (TLC) using pre-coated silica gel plates (Wako-gel B-5: Wakogel B-5FM = 4 : 1, 0.5 mm thickness, 20 × 20 cm) and a solvent system of $CHCl_3$–acetone (40 : 1). A band at *Rf* 0.71 was scraped off under an ultraviolet (UV) lamp, and extracted twice with 30 ml of $CHCl_3$. Then, the extract was evaporated under N_2, and the residue was subjected to silica gel preparative TLC using a solvent system of benzene–ethylacetate (19 : 1). A band at *Rf* 0.22 was removed under a UV lamp and extracted with $CHCl_3$ (30 ml × 2). The compound thus obtained was confirmed to be 8-oxo-Δ^9-THC on the basis of infrared (IR), mass (MS) and proton nuclear magnetic resonance (^{1}H-NMR) spectra. Yield was 101 mg (63.2% from 1-acetyl-8-oxo-Δ^9-THC). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1680 (C=O). MS *m/z*: 328 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (t, ω-CH_3), 1.17 (s, C(6α)-CH_3), 1.38 (s, C(9)-CH_3), 1.81 (s, C(6β)-CH_3), 3.51 (dd, J=15 Hz, J=5 Hz, C(10a)-H), 6.20 (d, J=2 Hz, C(2)-H), 6.30 (d, J=2 Hz, C(4)-H), 7.81 (br s, C(10)-H).

Drugs and Animals——Male ddN mice (23—25 g body weight) were purchased from Hokuriku Experimental Animals Lab. (Kanazawa, Japan). Pentobarbital-Na was obtained from Mallinckrodt Chemical Works (St. Louis, MO., U.S.A.). It was dissolved in isotonic saline (40 mg/10 ml) and injected into mice intraperitoneally (*i.p.*) in a volume of 0.1 ml/10 g of body weight (40 mg/kg). Cannabinoids were dispersed in isotonic saline containing 1% Tween 80, and injected into mice intravenously (*i.v.*, 0.1 ml/10 g of body weight). Animal experiments were carried out in an air-conditioned room (23 ± 1 °C).

Catalepsy——Three groups of mice (each n=40) were injected with cannabinoid at various doses. Cataleptogenic effect was assessed 15 min after the injections by the simple bar test reported before.[13]

Hypothermia——Five groups of mice (each n=10) were given the synthetic metabolites (5 and 10 mg/kg), Δ^9-THC (5 mg/kg) or vehicle (1% Tween 80-saline). Rectal temperature was measured at various time intervals up to 2 h after injection by means of a thermister probe attached to an electric thermometer (Natsume Seisakusho, Co., Tokyo, Japan).

Pentobarbital-Induced Sleep Prolongation——Eight groups of mice (each n=10) were injected with Δ^9-THC (5 mg/kg), 8α-OH-, 8β-OH- and 8-oxo-Δ^9-THCs (5 and 10 mg/kg) and the vehicle. Pentobarbital-Na (40 mg/kg) was administered 20 min after the above injection. Time intervals between loss and regaining of righting reflex were recorded as sleeping times.

Statistical Analysis——In the case of cataleptogenic effect, ED_{50} values and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon.[14] Statistical significance was analyzed by using Student's *t*-test.

Results and Discussion

ED_{50} values and their 95% confidence limits for catalepsy are listed in Table I. The values for 8β-OH-Δ^9-THC (4.3 mg/kg) is 1.6 times larger than that for Δ^9-THC (2.6 mg/kg). Only one of 8 mice treated with 8α-OH-Δ^9-THC showed a positive response even at a dose of 8.3 mg/kg. In the 8-oxo-Δ^9-THC-injected group, no mice exhibited catalepsy at a dose of 8.3 mg/kg, and two of 8 treated mice showed a positive response at a dose of 20.0 mg/kg.

Figure 1 shows the hypothermic effect produced by the cannabinoids. None of the

TABLE I. Cataleptogenic Effect in the Mouse of Δ^9-THC and Its Metabolites Oxidized at the 8-Position

Cannabinoids	n[a]	ED_{50} (mg/kg, *i.v.*)
Δ^9-THC[b]	8	2.6 (1.6—4.3)[c]
8α-OH-Δ^9-THC	8	>8.3
8β-OH-Δ^9-THC	8	4.3 (2.7—7.0)[c]
8-oxo-Δ^9-THC	8	>20.0

a) Number of mice per group.
b) The value is taken from our previous report.[15]
c) 95% Confidence limits.

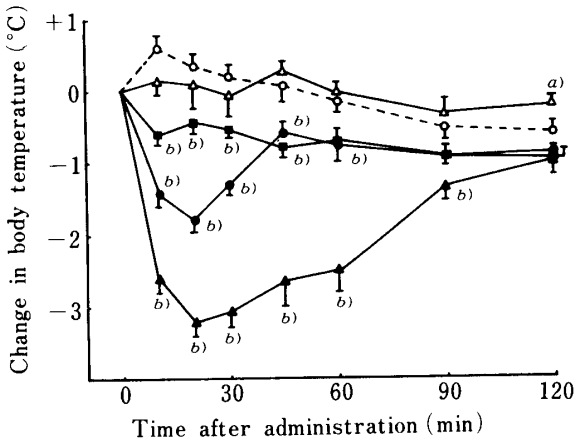

Fig. 1. Hypothermic Effect in the Mouse of Δ^9-THC and Its Metabolites Oxidized at the 8-Position

Rectal temperatures just before the treatments (0 min) were 37.6±0.3, 37.5±0.4, 37.6±0.4, 37.8±0.3 and 37.6±0.4 °C in the Δ^9-THC-, 8α-OH-Δ^9-THC-, 8β-OH-Δ^9-THC-, 8-oxo-Δ^9-THC- and vehicle (1% Tween 80 saline)-injected groups, respectively. Each point represents the mean value±S.E. of 10 mice. ▲—▲, Δ^9-THC (5 mg/kg); ●—●, 8α-OH-Δ^9-THC (10 mg/kg); △—△, 8β-OH-Δ^9-THC (10 mg/kg), ■—■, 8-oxo-Δ^9-THC (10 mg/kg); ○---○, control. *a*) Significantly different from the control ($p<0.05$). *b*) Significantly different from the control ($p<0.01$).

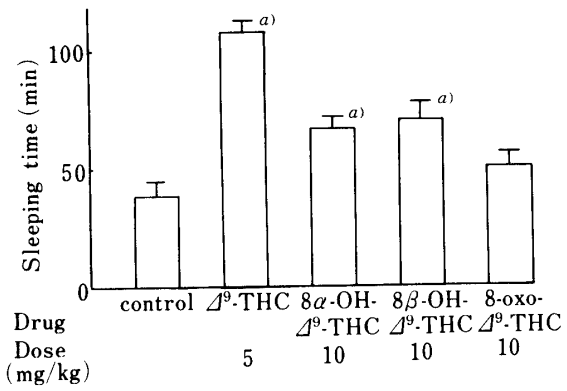

Fig. 2. Pentobarbital-Induced Sleep-Prolonging Effect in the Mouse of Δ^9-THC and Its Metabolites Oxidized at the 8-Position

Pentobarbital-Na (40 mg/kg, *i.p.*) was administered to mice 20 min after injection of cannabinoids or vehicle (1% Tween 80–saline). Each bar represents the mean value±S.E. of 10 mice. *a*) Significantly different from the control ($p<0.01$).

metabolites oxidized at the 8-position showed a significant effect on the rectal temperature of mice at a dose of 5 mg/kg, while the parent compound lowered the temperature significantly (−3.2 °C at 30 min after injection) as compared with the control group. On increasing the dose to 10 mg/kg, 8α-OH- and 8-oxo-Δ^9-THCs significantly lowered the rectal temperature, but 8β-OH-Δ^9-THC was still ineffective.

The effect of the cannabinoids on pentobarbital-induced sleep is shown in Fig. 2. At a dose of 5 mg/kg, the three metabolites did not significantly affect the sleeping time whereas Δ^9-THC prolonged the sleeping time 2.8-fold (108±5 min) as compared with the control (39±7 min). 8α-OH- and 8β-OH-Δ^9-THCs were found to have almost the same potency in barbiturate synergism at 10 mg/kg. The sleeping times were 66±5 and 70±3 min, respectively, in the 8α-OH-Δ^9-THC- and 8β-OH-Δ^9-THC-treated groups. 8-Oxo-Δ^9-THC had no activity up to a dose of 10 mg/kg in this case.

The present study showed that the pharmacological activities of the metabolites were much less potent than that of Δ^9-THC in terms of all three indices used. Thus, the oxidation at the 8-position can be regarded as one of the detoxication pathways of Δ^9-THC. We recently found that the pharmacological activity of 7-oxo-Δ^8-THC is comparable to that of Δ^8-THC in the mouse.[16] These findings are interesting in connection with the structure–activity relationship of cannabinoids. It is also of interest that the relative activities of 8α- and 8β-OH-Δ^9-THCs varied in each pharmacological index. Namely, the potency of 8α-OH-Δ^9-THC is lower in catalepsy, higher in hypothermia, and equal in barbiturate synergism as compared with that of 8β-OH-Δ^9-THC. These results suggest considerable complexity of cannabinoid action in the central nervous system.

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