3,4,5-Trimethoxyphenylacetaldehyde, an Intermediate Metabolite of Mescaline, Is a Substrate for Microsomal Aldehyde Oxygenase in the Mouse Liver

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3,4,5-Trimethoxyphenylacetaldehyde, an intermediate metabolite of mescaline, was oxidized to 3,4,5-trimethoxyphenylacetic acid by mouse hepatic microsomes. The reaction was NADPH-dependent, and inhibited by SKF 525-A, metyrapone and disulfiram. A P450 isozyme in mouse hepatic microsomes, P450 MUT-2 (CYP2C29), catalyzed the reaction (0.96 nmol/min/nmol P450) in which NADPH and NADPH-cytochrome c reductase were essential for the catalytic activity. The reaction was confirmed to be an oxygenation since molecular oxygen was incorporated into the carboxylic acid metabolite formed under oxygen-18 gas by GC-MS analysis. By addition of antibody against CYP2C29 to the microsomes (3.2 mg/mg microsomal protein) the MALDO activity was inhibited by 35% of the control value with preimmune serum, suggesting that CYP2C29 or an immunologically-related isozyme(s) plays a major role in the NADPH-dependent oxidation of 3,4,5-trimethoxyphenylacetaldehyde to 3,4,5-trimethoxyphenylacetic acid by mouse hepatic microsomes. Pharmacological experiments on mescaline and its deaminated metabolites using mice indicated that the metabolites were much less active or were inactive in cataleptogenic effect and pentobarbital-induced sleep prolongation as compared with the parent compound.

Key words microsomal aldehyde oxygenase; P450; mescaline; 3,4,5-trimethoxyphenylacetaldehyde; oxygenation; microsome

Many studies have been reported on the metabolism of mescaline, β -(3,4,5-trimethoxyphenyl)-ethylamine known to be a psychoactive component of peyote.¹⁾ The *in vivo* major metabolic route is *via* oxidative deamination to 3,4,5-trimethoxyphenylacetic acid (TMPA) through 3,4,5-trimethoxyphenylacetaldehyde (TMPAld) in various animal species including humans. In the reaction, the copper-containing plasma form of amine oxidase but not mitochondrial monoamine oxidase is suggested to be involved.^{1c,2)} Aldehyde dehydrogenase might be responsible for the formation of TMPA since *in vivo* formation of 3,4,5-trimethoxyphenylethanol (TMPE) was increased in rats by the treatment with calcium carbamide.³⁾ This metabolic route is very important for understanding pharmacological effects of mescaline because Friendhoff and Goldstein⁴⁾ suggested that TMPAld or TMPE is related to the psychoactivity.

Our recent studies demonstrated that microsomal aldehyde oxygenase (MALDO) could metabolize some xenobiotic aldehydes,⁵⁾ although no evidence is available concerning aldehyde substrates formed from amines by oxidative deamination. The present study thus describes the metabolism of TMPAld to TMPA by mouse hepatic MALDO. Pharmacological effects of mescaline and its deaminated metabolites are also evaluated.

MATERIALS AND METHODS

Chemicals Mescaline hydrochloride was synthesized by the method described by Baxter *et al.*⁶⁾ TMPE was prepared by LiAlH₄ reduction of TMPA. ¹H-NMR of TMPE (CDCl₃), δ : 6.29 (2H, s, C₂-H and C₆-H), 3.75 (9H, s, tri-OCH₃), 2.74 (2H, t, –CH₂–). MS *m/z*: 212 (M⁺, 49%), 181 (100%). TMPAld was synthesized by CrO₃

oxidation of TMPE by the method of Ratcliffe and Rodehorst.⁷⁾ ¹H-NMR of TMPAld (CDCl₃), δ : 9.42 (1H, t, –CHO), 6.25 (2H, s, C₂-H and C₆-H), 3.79 (9H, s, tri-OCH₃), 3.52 (2H, d, –CH₂–). MS *m/z*: 210 (M⁺, 45%), 181 (100%). TMPA was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.); NAD, NADP, NADH and glucose-6-phosphate were from Boehringer-Mannheim GmbH (Darmstadt, F.R.G.); NADPH was from Oriental Yeast Co. (Tokyo, Japan); metyrapone, dilauroylphosphatidylcholine and glucose-6-phosphate dehydrogenase (type V) were from Sigma Chemical Co. (St Louis, MO, U.S.A.); disulfiram and sodium barbital were from Wako Pure Chem. Ind. (Osaka, Japan); pyrazole was from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). SKF 525-A was kindly supplied by Smith Kline and French Lab. (Philadelphia, PA, U.S.A.). Oxygen-18 gas (95% atom) was purchased from CIL (Woburn, MA, U.S.A.).

Enzyme Preparation Six week-old male ddN mice (25–35 g) were obtained from Hokuriku Experimental Animal Lab. (Kanazawa, Japan). Hepatic microsomes were prepared by different centrifugation force as described previously.⁸⁾ P450 MUT-2 (CYP2C29)^{5c,9)} and NADPH-cytochrome c reductase¹⁰⁾ were purified from the microsomes by the reported methods. Antiserum against purified CYP2C29 was prepared in female New Zealand white rabbits as described previously.⁹⁾

Enzyme Assays Hepatic microsomal metabolism of TMPAld was carried out under the following conditions. The typical incubation mixture consisted of microsomes (0.1 g liver equivalent), 0.5 mM NADP, 10 mM glucose-6-phosphate, 10 mM MgCl₂, 1 unit glucose-6-phosphate dehydrogenase, 500 nmol TMPAld and 100 mM sodium potassium phosphate buffer (pH 7.4) to make a final volume of 1 ml. The mixture was incubated at 37 °C for

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30 min and the reaction was terminated by addition of 0.5 ml of 0.2 M KCl-HCl and 2 μ g of 3,5-dimethoxybenzoate as the internal standard. After extraction with 4 ml of ethyl acetate and evaporation of the solvent, TMPA formed was methylated by addition of CH_2N_2 ethereal solution. The methyl ester was analyzed by GC-MS using a JEOL-GCG-06 gas chromatograph coupled with a JEOL JMS-DX 300 mass spectrometer and a JEOL-DA 5000 mass data system. The conditions were as follows: column, 2% OV-17 on Chromosorb W (60–80 mesh, 3 mm $\times$ 2 m); column temperature, 200 $^\circ\text{C}$; carrier gas, He 40 ml/min; ionizing current, 300 μamp .; ionizing energy, 70 eV. The retention time of methyl ester of TMPA was 5.9 min under the above conditions.

The reconstituted system was constructed by mixing 49.8 pmol of CYP2C29 with 0.33 U of NADPH-cytochrome c reductase, 15 μ g of dilauroyl phosphatidylcholine, 500 nmol of NADPH and 400 nmol of TMPA1d in 100 mM sodium potassium phosphate buffer (pH 7.4) to make a final volume of 0.5 ml. The reaction was started by addition of the cofactor and TMPA formed was analyzed as described above. The incubation under oxygen-18 gas was carried out as described previously.^{5a,b)} A reaction mixture consisted of the same system as described above except that hepatic microsomes equivalent to 0.3 g liver were used and incubation atmosphere was replaced by oxygen-18 gas (97 atom%). The inhibition study on antibody was performed in the 0.5 to 3.2 mg serum protein/mg microsomal protein as described previously.⁹⁾ P450 and protein in microsomes were determined by the method of Omura and Sato,¹¹⁾ and Lowry *et al.*,¹²⁾ respectively. NADPH-cytochrome c reductase was assayed by the method of Phillips and Langdon.¹³⁾

Pharmacological Experiments Pharmacological effects of mescaline and its deaminated metabolites, TMPE, TMPA1d and TMPA were evaluated using male ddN mice (20–25 g, 5 weeks-old), and catalepsy (each group, $N=8$) and pentobarbital-induced sleep prolongation (each group, $N=10$) as indices as described previously for assessing pharmacological effects of cannabinoids.¹⁴⁾ Mescaline hydrochloride was dissolved in physiological saline and its metabolites were suspended in saline containing 1% Tween 80. These compounds were administered i.v. through the tail vein (10 ml/kg body weight).

RESULTS

Microsomal Oxidation of TMPA1d TMPA1d was oxidized to TMPA with hepatic microsomes. NADPH (0.62 nmol/min/mg protein) was the most effective cofactor followed by NAD (0.25 nmol/min/mg protein) (Fig. 1). NADP and NADH were less active in this role. TMPA was not formed when these cofactors were not added to the incubation mixture. The NADPH-dependent formation of TMPA was inhibited by SKF 525-A, metyrapone and disulfiram (1 mM), while pyrazole and barbitol did not affect the reaction at all (Table 1). These characteristics are similar to that of MALDO activity for other substrates.¹⁵⁾

Mass chromatograms and the mass spectrum of TMPA

Fig. 1. Cofactor Requirement for Hepatic Microsomal Oxidation of TMPA1d

Each bar represents the mean of duplicate incubations.

Table 1. Effects of Various Inhibitors on NADPH-Dependent Formation of TMPA with Mouse Hepatic Microsomes

Addition ^{a)}	% of control	Addition	% of control
Control ^{b)}	100	SKF 525-A	10
Pyrazole	92	Metyrapone	24
Barbital	96	Disulfiram	6

a) Inhibitor added was 1 mM each. b) Specific activity in the control was 1.34 nmol/min/mg protein. The data represent the mean of duplicate incubations.

Fig. 2. Mass Chromatograms and Mass Spectrum of TMPA Formed with Mouse Hepatic Microsomes under Oxygen-18 Gas

m/z 240:242=100:54 TIC: total ion chromatogram.

formed with the microsomes under oxygen-18 gas are shown in Fig. 2. The molecular ions of methylated TMPA appeared at m/z 240 and 242 and relative abundance was 100:54, showing incorporation of molecular oxygen into TMPA.

Contribution of P450 to TMPA Formation CYP2C29 catalyzed the oxidation of TMPA1d to TMPA in which NADPH and NADPH-cytochrome c reductase were

Table 2. TMPA Forming Activity with Reconstituted System Containing CYP2C29

	Specific activity (nmol/min/nmol P450)
Complete system ^{a)}	0.96
+ cytochrome b ₅	1.24
– CYP2C29	ND
– NADPH–cytochrome c reductase	ND
– NADPH	ND

a) The incubation system consisted of 49.8 pmol CYP2C29, 0.33 U NADPH–cytochrome c reductase, 15 μ g of dilauroylphosphatidylcholine and 400 nmol of TMPAld in 0.5 ml of 100 mM phosphate buffer (pH 7.4). ND: The activity was not detectable. The data represent the mean of duplicate incubations.

Fig. 3. Inhibitory Effect of Anti-CYP2C29 Serum on Hepatic Microsomal Oxidation of TMPAld to TMPA

○—○, control serum; ●—●, anti-CYP2C29 serum. Each point represents the mean of duplicate incubations.

essential (Table 2). The addition of cytochrome b₅ increased the activity about 30%. The results suggest that CYP2C29 may be responsible for the MALDO activity in mouse hepatic microsomes. Thus, the effect of antibody against CYP2C29 on the MALDO activity was determined (Fig. 3). The addition of preimmune control rabbit serum did not affect the MALDO activity at all. When anti-CYP2C29 serum was added to the incubation mixture at the relative concentration of 0.5 to 3.2 mg/mg microsomal protein, the MALDO activity was inhibited by 42% to 35% as compared with the activity without the rabbit serum.

Pharmacological Effects Pharmacological effects of mescaline and its metabolites including TMPAld were evaluated using catalepsy and pentobarbital-induced sleep prolongation as indices. ED₅₀ (mg/kg, i.v. and 95% confidence limits) of mescaline was 44.0 (41.0–47.4) for the cataleptogenic effect. No positive cataleptogenic effect was observed by TMPE or TMPA at a dose up to 100 mg/kg i.v. while TMPAld exhibited this effect to some extent (25%) at the same dose.

Mescaline dose-dependently prolonged pentobarbital (50 mg/kg, i.p.)-induced sleeping time. At doses of 25 and 50 mg/kg i.v., prolongation was significant ($p < 0.01$, by ANOVA), by 4.5 and 5.3 fold, respectively, as compared with the control mice (43 ± 4 min, $N = 10$). None of TMPE, TMPAld or TMPA significantly prolonged the sleeping time even at doses of 50 or 100 mg/kg, i.v.

DISCUSSION

TMPAld, a key intermediate in the metabolism of mescaline, was oxidized to a corresponding carboxylic acid, TMPA by mouse hepatic microsomes in which NADPH was the most effective cofactor and NAD was less effective. Our previous work demonstrated that a similar cofactor requirement was observed in the hepatic microsomal oxidation of tolualdehydes.¹⁶⁾ On the other hand, NADPH was an obligate cofactor in the reaction of 11-oxo- Δ^8 -tetrahydrocannabinol^{15a)} and 9-anthraldehyde^{15b)} as substrates. The differences may thus be due to substrate-specificity of the two enzymes, NAD-dependent dehydrogenase and NADPH-dependent MALDO.

The NADPH-dependent oxidation of TMPAld to TMPA was significantly inhibited by SKF 525-A, metyrapone and disulfiram, indicating that P450 was responsible for the reaction. The lack of inhibition by pyrazole or barbitol suggests that neither alcohol dehydrogenase nor aldehyde reductase mediate the reaction. A significant incorporation of molecular oxygen into the molecule of TMPA formed during NADPH-dependent oxidation of TMPAld with mouse hepatic microsomes indicated that MALDO is the main contributor to the reaction, although other mechanisms may be involved to some extent since the incorporation of molecular oxygen was incomplete. The relative contribution of oxygenation mechanism to the microsomal oxidation of TMPAld was the same degree as that in cuminaldehyde reported previously.^{5b)}

Mouse CYP2C29 has been reported to be a major isozyme catalyzing the oxidation of 11-oxo- Δ^8 -tetrahydrocannabinol⁹⁾ and tolualdehyde¹⁶⁾ in the mouse liver. It is known that rat CYP2C11 is a major isozyme responsible for the MALDO activity of 11-oxo- Δ^8 -tetrahydrocannabinol¹⁷⁾ and 9-anthraldehyde^{5d)} in male rat liver. The present study demonstrated that CYP2C29 or immunologically-related isozyme(s) is the main contributor to the MALDO activity for TMPAld in the mouse liver. Incomplete inhibition by the antibody against CYP2C29 on the MALDO activity suggests that other isozyme(s) may also contribute to the activity.

In the pharmacological experiments using inhibitors of amine oxidase and aldehyde dehydrogenase, Friedhoff and Goldstein⁴⁾ suggested that mescaline metabolites, TMPAld and TMPE, were active metabolite candidates. In contrast, Smythies *et al.*¹⁸⁾ suggested that mescaline itself was active. The present study demonstrated that three deaminated metabolites of mescaline were not as active as mescaline when cataleptogenic effect and pentobarbital-induced sleep prolongation were used as indices. Rapid metabolism of these metabolites also suggests that they play only a minor role in the pharmacological effect of mescaline. Further experiments using other pharmacological indices are necessary to clarify the participation of these metabolites in the pharmacological effects of mescaline since TMPAld exhibited a cataleptogenic effect to some extent.

The present study indicates that TMPA is a good substrate for MALDO in the mouse liver, and suggests

that aldehydes derived from biologic amines may also be substrates for the enzyme. This suggestion should confirm the additional role of MALDO from pharmacological and toxicological points of view.

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