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 (CYP2C9), catalyzed the reaction (0.96 mmol/mg/min of 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. In addition of antibody against CYP2C9 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 CYP2C9 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 (TMPA1d) 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.1,2) Aldehyde dehydrogenase might be responsible for the formation of TMPA since in vivo formation of 3,4,5-trimethoxyphenylethanol (TMPA) was increased in rats by the treatment with calcium carbamidc.3) This metabolic route is very important for understanding pharmacological effects of mescaline because Friendhoff and Goldstein4) suggested that TMPA1d 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 TMPA1d 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. ³H-NMR of TMPE (CDCl₃, δ: 6.29 (2H, s, C₆-H and C₅-H), 3.75 (9H, s, tri-OCH₃), 2.74 (2H, t, −CH₂−). MS m/z: 212 (M⁺, 49%), 181 (100%). TMPA1d was synthesized by CrO₃ oxidation of TMPE by the method of Ratcliff and Rodehorst.7) ³H-NMR of TMPA1d (CDCl₃, δ: 9.42 (1H, t, −CHO), 6.25 (2H, s, C₆-H and C₅-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, diluoroarylphosphatidylcholine 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 (CYP2C9) and NADPH-cytochrome c reductase were purified from the microsomes by the reported methods. Antiserum against purified CYP2C9 was prepared in female New Zealand white rabbits as described previously.9)

Enzyme Assays Hepatic microsomal metabolism of TMPA1d was carried out under the following conditions. The typical incubation mixture consisted of microsomes (0.1 g liver equivalent), 0.5 mM NADPH, 10 mM glucose-6-phosphate, 10 mM MgCl₂, 1 unit glucose-6-phosphate dehydrogenase, 500 nmol TMPA1d 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 μg 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 CH3N2, 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 x 2 m); column temperature, 200 °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 CYP2C9 with 0.33 U of NADPH-cytochrome c reductase, 15 μg of dilauroyl phosphatidylcholine, 500 nmol of NADPH and 400 nmol of TMPAid in 100 mM 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. 2a,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. 3a P450 and protein in microsomes were determined by the method of Omura and Sato, 9 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 degraded metabolites, TMPE, TMPAid 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 TMPAid TMPAid 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 barbital 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 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 CYP2C9 catalyzed the oxidation of TMPAid to TMPA in which NADPH and NADPH-cytochrome c reductase were
Table 2. TMPA Forming Activity with Reconstituted System Containing CYP2C9

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Specific activity (nmol/min/nmol P450)</th>
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<tbody>
<tr>
<td>Complete system</td>
<td>0.96</td>
</tr>
<tr>
<td>+ cytochrome b$_5$</td>
<td>1.24</td>
</tr>
<tr>
<td>- CYP2C9</td>
<td>ND</td>
</tr>
<tr>
<td>- NADPH-cytochrome c reductase</td>
<td>ND</td>
</tr>
<tr>
<td>- NADPH</td>
<td>ND</td>
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a) The incubation system consisted of 49.8 pmol CYP2C9, 0.33 U NADPH-cytochrome c reductase, 15 µg of dilauroylphosphatidylcholine and 400 nmol of TMPA1d 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.

DISCUSSION

TMPA1d, 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. On the other hand, NADPH was an obligate cofactor in the reaction of 11-oxo-A4-tetrahydrocannabinol and 9-anthraldehyde 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 TMPA1d 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 barbital suggests that neither alcohol dehydrogenase nor aldehyde reductase mediated the reaction. A significant incorporation of molecular oxygen into the molecule of TMPA formed during NADPH-dependent oxidation of TMPA1d 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 TMPA1d was the same degree as that in cuminaldehyde reported previously.

Mouse CYP2C9 has been reported to be a major isozyme catalyzing the oxidation of 11-oxo-A4-tetrahydrocannabinol and tolualdehyde in the mouse liver. It is known that rat CYP2C11 is a major isozyme responsible for the MALDO activity of 11-oxo-A4-tetrahydrocannabinol and 9-anthraldehyde in male rat liver. The present study demonstrated that CYP2C9 or immunologically-related isozyme(s) is the main contributor to the MALDO activity for TMPA1d in the mouse liver. Incomplete inhibition by the antibody against CYP2C9 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 Goldstein suggested that mescaline metabolites, TMPA1d and TMPE, were active metabolite candidates. In contrast, Smythies et al. 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 TMPA1d 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.

Acknowledgments We thank Mrs. R. Igarashi for carrying out GC-MS analyses. A part of the present study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and by the Special Research Fund of Hokuriku University.

REFERENCES


