Suppression of Liver Microsomal Drug-Metabolizing Enzyme Activities in Adult Female Rats Pretreated with Cannabidiol

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The suppression by cannabidiol (CBD) of the liver microsomal drug-metabolizing enzyme activities in female rats was demonstrated and its mechanism was examined. Pretreatment of rats with CBD (10 mg/kg, p.o.) caused temporary decreases in contents of cytochrome P450 (P450) and b, and NADPH-cytochrome c (P450) reductase activity compared with values from the vehicle control group. p-Nitroanisole O-demethylase, aniline hydroxylase, d-benzphetamine N-demethylase and Δ⁹-tetrahydrocannabinol 11-hydroxylase were also decreased by the CBD pretreatment. The latter two activities took a longer time to return to control levels than the former two. However, the CBD pretreatment, which reduced the protein level of P450 UT-2 (CYP2C11) in adult male rats, did not decrease the protein level of P450 F-1 (CYP2C6) or F-2 (CYP2C12) in liver microsomes from female rats. These results suggest that the mechanisms by which CBD suppresses liver microsomal drug-metabolizing enzyme activities are different in male and female rats.

Keywords: cannabidiol; rat liver microsome; suppressive activity; cytochrome P450; d-benzphetamine; Δ⁹-tetrahydrocannabinol

Introduction

Cannabidiol (CBD), one of the major constituents of marihuana, is known to decrease liver microsomal drug metabolism in mammals. The mechanism involved has been investigated, but has not yet been established. We recently reported that, in liver microsomes of adult male rats pretreated with CBD, one of the mechanisms causing the decreased activity is a reduction of oxidation activity and the level of cytochrome P450 (P450) UT-2 (CYP2C11), a male-specific P450 isozyme. However, we observed in preliminary experiments that pretreatment of female rats with CBD also caused decreases in oxidation activities for aniline and p-nitroanisole in liver microsomes. It occurred to us that the suppression of the drug metabolism in adult female rats might be caused by a mechanism different from that in adult male rats, since the male-specific P450 isozyme is absent in liver microsomes of adult female rats. This paper characterizes the mechanism by which CBD reduces drug metabolism enzyme activities in female rat liver microsomes.

Materials and Methods

General Glucose 6-phosphate (G-6-P), NADP and NADPH were purchased from the Boehringer-Mannheim GmbH (Darmstadt, Germany); G-6-P dehydrogenase (type V, EC 1.1.1.49), molecular weight marker (Dalton Mark VII) for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), cytochrome c (type III from horse heart) and dialauroylphosphatidylethanoline were all from the Sigma Chemical Co. (St. Louis, MO, U.S.A.); anti-rabbit IgG conjugated with alkaline phosphatase was from ICN Immuno-Biologicals (Lisle, IL, U.S.A.). Freund’s complete adjuvant was from the Wako Pure Chemicals Co. (Osaka, Japan); a membrane for electrophoresis (Clear Blot Membrane-p) was from the Atto Co. (Tokyo, Japan). CBD and Δ⁹-tetrahydrocannabinol (THC) were purified from cannabis leaves by the method previously reported. 1-Hydroxy-Δ⁹-THC (11-OH-Δ⁹-THC) was supplied from the National Institutes on Drug Abuse (Bethesda, MD, U.S.A.). Other reagents and solvents used were of the highest quality commercially available.

Treatment of Rats with CBD Adult female Sprague-Dawley rats (9 weeks-old) were given CBD (10 mg/kg, i.p., in saline containing 1% Tween 80), and killed by decapitation 3, 6, 12, 24, 48 and 72 h later. Liver microsomes were prepared by the method reported previously.

Assay of Oxidation Activities The activity of NADPH-cytochrome c (P450) reductase was measured by the method of Phillips and Langdon, 12 while the levels of P450 and cytochrome b5 were determined by the methods of Omura and Sato. 13 Liver microsomal oxidation activities toward aniline (AN), p-nitroanisole (p-NA), d-benzphetamine (d-BP) and Δ⁹-THC 13 were assayed according to published methods. Protein concentration was measured by the Lowry method. 14

Others P450 F-1 (CYP2C6) and F-2 (CYP2C12) were purified from liver microsomes of adult female rats as reported previously. 15 Antiserum against P450 F-1 and F-2 were obtained by immunizing rabbits with purified enzymes as reported previously. 16 SDS-PAGE and Western blot analysis were performed by the methods of Laemmli 17 and Gonzalez et al. 18 respectively, using 8% polyacrylamide slab-gels. Statistical significance was assessed by Student’s t-test.

Results and Discussion

Figure 1 depicts the effect, following a single administration of CBD to female rats, on the drug-metabolizing enzyme system in the liver microsomes. Compared with the values of the control group which received a vehicle (0.9% NaCl containing 1% Tween 80), the P450 contents and NADPH-cytochrome c (P450) reductase activities decreased significantly over the period 6 to 24 h after the injection. These reduced levels returned to the control levels 48 h later. Cytochrome b5 levels were significantly lower than the controls 6 h after injection (Fig. 1).

AN hydroxylase, p-NA O-demethylation and d-BP N-demethylation were measured as indices of the drug-metabolizing enzyme activities (Fig. 1). All the activities examined were suppressed significantly as early as 3 h after cannabidiol treatment. p-NA O-demethylation returned to the control level most quickly, being followed by AN hydroxylase. The activity returning most slowly to the control level was d-BP N-demethylation, which needed 72 h to regain the normal activity.

We demonstrated recently that Δ⁹-THC, a major psychoactive component of marihuana, is biotransformed mainly into 11-OH-Δ⁹-THC in the liver microsomes of female rats. When microsomal oxidation of Δ⁹-THC was examined, 11-OH-Δ⁹-THC formation was markedly de-
creased in liver microsomes of CBD-treated female rats. In Fig. 2, a time course of 11-OH-\(\Delta^9\)-THC formation, after cannabinoïd injection, was compared with the P450 levels determined spectrophotometrically and with \(d\)-BP N-demethylation. The profile of 11-OH-\(\Delta^9\)-THC formation paralleled that of \(d\)-BP N-demethylation. Namely, the P450 returned to control levels within 48 h after CBD administration, but the 11-OH-\(\Delta^9\)-THC formation and \(d\)-BP oxidation remained at about 55 to 60% of the control levels even 48 h after treatment.

This phenomenon is very similar to that observed in male rats pretreated with CBD, i.e., various activities of liver microsomal drug-metabolizing enzymes were decreased.\(^9\) In the previous study,\(^9\) however, pretreatment of adult male rats with CBD caused a sustained reduction of a male-specific P450 UT-2 (CYP2C11) protein, which is a major isozyme catalyzing \(d\)-BP oxidation in the liver microsomes of adult male rats.\(^{22,23}\) We have demonstrated recently a considerable sex difference in the oxidative metabolism of \(\Delta^9\)-THC in rats both \textit{in vivo} and \textit{in vitro}.\(^{17}\)

When oxidation activities, with respect to \(\Delta^9\)-THC, of P450 isoforms purified from liver microsomes of adult female rats were examined in a reconstituted system, P450 F-1 (CYP2C6) exhibited 11-OH-\(\Delta^9\)-THC-forming activity exclusively, while P450 F-2 (CYP2C12), a female-specific isozyme, did not show any oxidative activity towards \(\Delta^9\)-THC, under the conditions used.\(^{24}\)

We have found that CBD pretreatment in male rats reduces levels of P450UT-2 (CYP2C11), and believed this to be one of the mechanisms causing the decrease by CBD of liver microsomal drug metabolism.\(^9\) Imaoka \textit{et al.}\(^{19}\) have reported that P450 F-1 (CYP2C6) immunochemically cross-reacted with P450 UT-2 (CYP2C11). From these findings and the present results, it is feasible that CBD might also decrease levels of P450 F-1 (CYP2C12) in female rats as is the case with P450 UT-2 (CYP2C11) in male rats. To examine the effects of CBD pretreatment on the P450 protein levels, we investigated the P450 isoforms in the liver microsomes of female rats, after they received a CBD injection, by Western blot analysis using antiserum against P450 F-1 (CYP2C6) or P450 F-2 (CYP2C12). The analysis revealed that pretreatment of female rats with CBD did not alter the levels of either P450 F-1 or P450 F-2 (data not shown).

In our previous study,\(^{24}\) we observed that antiserum against P450 F-1 (CYP2C6) suppressed 11-OH-\(\Delta^9\)-THC formation by liver microsomes from adult female rats in a concentration-dependent manner, while antiserum against P450 F-2 (CYP2C12) had no effect at all. These findings and the results obtained here indicate that, as observed in male rats, pretreatment of adult female rats with CBD causes a temporary reduction in the oxidation activities of AN and \(p\)-NA mediated by some liver microsomal P450 isoforms and a rather longer reduction in the oxidation activities of \(\Delta^9\)-THC and \(d\)-BP which were catalyzed by P450 F-1 (CYP2C6). Taking into account that pretreatment of female rats with CBD did not affect the levels of P450 F-1 (CYP2C6) or of P450 F-2 (CYP2C12) in liver microsomes,
it seems likely that CBD itself in the early stage, or some metabolite(s) of CBD in the later stage of the suppression, decreased hepatic drug metabolism either by interacting with a P450 isozyme(s), or by reducing the level of a P450 isozyme(s) other than P450 F-1 (CYP 2C6) or P450 F-2 (CYP 2C12). Miwa et al. have reported that NADPH–cytochrome c (P450) reductase could be a rate-limiting enzyme for the liver microsomal oxidation of some substrates such as d-BP and 7-ethoxycoumarine.25) Since CBD caused significant suppression of NADPH–cytochrome c (P450) reductase activities over the period 6 to 24 h after the injection (Fig. 1), it is possible that decreased reductase activity might cause, at least in part, the suppression of the microsomal oxidation activities of the substrates examined. However, this cannot explain the reduced oxidation activity of d-BP or Δ⁹-THC, assayed 48 h after the CBD pretreatment, when the activity of the reductase had returned to the control level.

Recently Deutsch et al.26) reported that in male rats, a messenger RNA of CYP2C7, a constitutive P450 isozyme inducible with phenobarbital, was slightly decreased by CBD pretreatment. At present, we do not know how important this phenomenon is in the mechanism by which CBD causes the suppression of liver microsomal drug metabolism. However, their study indicates that it is necessary to estimate the turnover rates of proteins and messenger RNA levels of constitutive P450 isozymes after CBD treatment in order to further investigate the mechanism(s) by which CBD suppresses liver microsomal drug metabolism and the sex-difference phenomena observed here.

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References and Notes
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