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Purification and Characterization of a Mouse Liver Cytochrome P-450 Induced by Cannabidiol

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SUMMARY

A cytochrome P-450 isozyme (M_r = 51,600) was purified to apparent homogeneity from hepatic microsomes of mice pretreated with cannabidiol (CBD), a major constituent of marijuana. The isozyme exhibited high pentoxyresorufin *O*-dealkylase, hexobarbital hydroxylase, and 16α- and 16β-testosterone hydroxylase activities and formed a Fe^{+2}-metyrapone complex, properties characteristic of the major hepatic cytochrome P-450s previously purified from phenobarbital (PB)-pretreated animals. In addition, the CBD-induced cytochrome P-450 was immunoreactive with an antibody raised against the major rat hepatic PB-inducible cytochrome P-450 and exhibited an NH_2-terminal amino acid sequence >90% homologous with that of the PB-inducible rat liver isozyme. Because of the many similarities between the CBD-induced isozyme and certain other isozymes previously purified from PB-pretreated animals, a cytochrome P-450 isozyme was purified from PB-pretreated mice by a chromatographic procedure similar to that employed for purification of the CBD-induced isozyme. The PB-inducible isozyme was indistinguishable from the CBD-inducible cytochrome P-450 on the bases of apparent molecular weight, absorption spectra, NH_2-terminal amino acid sequence, peptide mapping, immunoreactivity, and catalytic activity. Although the CBD- and PB-inducible P-450 isozymes appear to be qualitatively very similar, PB appears to be a quantitatively better inducer of the isozyme. Thus, CBD exposure results in the induction of an isozyme that is refractory to CBD-mediated inactivation, thereby apparently altering the cytochrome P-450 isozymal composition of mouse hepatic microsomes.

CBD is a major constituent of marijuana that has been shown to inhibit hepatic drug metabolism after acute treatment (1–9) and to induce metabolism after repetitive treatment in mice (9). CBD is probably ingested in quantities sufficient to affect drug metabolism in humans (10), through illicit consumption of marijuana, which may contain CBD in quantities >5% of the total dry weight (11). Clinically, CBD has also been shown to be an effective antiepileptic agent (12) when administered in even higher doses (200 mg). Although acute CBD treatment has been shown to markedly inhibit both *in vivo* and *in vitro* hexobarbital hydroxylase activity in the mouse, repetitive CBD treatment resulted in an apparent resistance to the inhibitory effect (9). Concurrent with the observed resistance to hexobarbital hydroxylase inhibition, a marked increase in PTR activity is found, with the appearance of a polypeptide ($M_r \approx$ 50,000) that is immunoreactive to an antibody prepared against the major rat hepatic P-450 induced by PB (13–15). These findings suggested that the resistance to CBD-mediated inhibition might be due to the induction of a P-450 isozyme not originally present in hepatic microsomes. Therefore, the purpose of this study was to attempt to isolate and purify this CBD-inducible P-450 ($P\text{-}450_{CBD}$) in order to identify and characterize it and elucidate the effect of its induction on hepatic drug metabolism.

CBD-mediated alterations in drug metabolism would be expected to affect the metabolism of THC (Fig. 1), the major psychoactive cannabinoid found in marijuana (16), as well as the metabolism of other drugs taken concurrently for either therapeutic or illicit purposes. THC has been shown to be extensively metabolized by P-450 and over 100 metabolites have been identified (17). Because the pharmacological activity of marijuana has been attributed not only to THC but also to its major metabolites (18, 19), any compound capable of altering THC metabolism might result in important pharmacological effects.

Experimental Procedures

Materials. CBD was generously supplied by the National Institute on Drug Abuse. DEAE- and CM-cellulose were purchased from Whatman (Kent, England), DEAE-Sephacel, sodium cholate, and Lubrol

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ABBREVIATIONS: CBD, cannabidiol; PTR, pentoxyresorufin *O*-dealkylase; P-450, cytochrome P-450; THC, Δ^9-tetrahydrocannabinol; PB, phenobarbital; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; $P\text{-}450_b$, the major rat hepatic isozyme purified from phenobarbital-pretreated rats, also referred to as PB-4 or PB-B; $P\text{-}450_{CBD}$, hepatic P-450 isozyme purified from cannabidiol-pretreated mice; $P\text{-}450_{PB}$, hepatic P-450 isozyme purified from phenobarbital-pretreated mice.

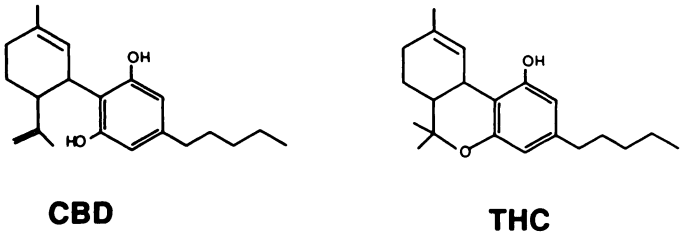
PX from Sigma (St. Louis, MO), and hydroxylapatite (Bio-Gel HTP) from BioRad (Richmond, CA). All other chemicals were of reagent grade.

Animals and treatment. Male CF-1 mice (Charles River, Portage, MI) weighing 20–30 g were used in all experiments. CBD was administered intraperitoneally in a Tween 80 suspension as described previously (5), at an anticonvulsant dose of 120 mg/kg once daily for 4 days, and mice were killed 24 hr after the last dose. Sodium phenobarbital was injected intraperitoneally, at a dose of 100 mg/kg, daily for 4 days.

P-450 purification. Animals were killed by cervical dislocation, the gall bladders were removed, and livers were perfused *in situ* with ice-cold 1.15% KCl. Livers were removed and microsomes were prepared as described previously (9). Microsomes were solubilized at 4° for 1 hr, at a final protein concentration of 10 mg/ml, in buffer containing 10 mM potassium phosphate, pH 7.4, 20% (v/v) glycerol, 0.5% (w/v) sodium cholate, 0.2% (v/v) Lubrol PX, and 0.1 mM EDTA (buffer A). Solubilized microsomes were applied at room temperature to a Whatman DEAE-cellulose (DE-52) column (10 nmol of P-450/ml of resin) equilibrated with buffer A. After the column was washed with buffer A (1 column volume), most of the P-450 was eluted with a linear gradient of 0–100 mM sodium chloride in buffer A (5 column volumes). Analysis by SDS-PAGE of the P-450-containing fractions revealed four major proteins of approximately 50 kDa, only one of which was immunoreactive after Western immunoblotting with an antibody raised against P-450$_b$. Fractions containing this immunoreactive protein and exhibiting $A_{417}/A_{280} \geq 0.4$ were pooled, concentrated, and dialyzed against buffer containing 10 mM sodium phosphate, pH 6.8, 20% (v/v) glycerol, 0.2% (v/v) Lubrol PX, and 0.1 mM EDTA (buffer B), before application to a Whatman CM-cellulose (CM-52) column (10 nmol of P-450/ml of resin) that was equilibrated in the same buffer. After washing with buffer B (1 column volume), P-450 was eluted with a linear gradient of 0–100 mM potassium phosphate, pH 6.8, in buffer B (5 column volumes). Fractions exhibiting high anti-P450$_b$ immunoreactivity and $A_{417}/A_{280} \geq 0.8$ were pooled, concentrated, and dialyzed against buffer containing 20 mM Tris, pH 7.7, 20% (v/v) glycerol, 0.2% (v/v) Lubrol PX, and 0.1 mM EDTA (buffer C), before application to a DEAE-Sephacel column (10 nmol of P-450/ml of resin) that was equilibrated in the same buffer. After washing with buffer C (2 column volumes), P-450 was eluted with a linear gradient of 0–100 mM sodium acetate, pH 7.7, in buffer C (10 column volumes) and was judged to be homogeneous and greater than 95% pure by SDS-PAGE. Nonionic detergent was removed as described previously (14), except that Lubrol PX was used in place of Emulgen 911, and P-450 was eluted from the hydroxylapatite column with buffer containing 0.1% (w/v) sodium cholate and 400 mM potassium phosphate. P-450 was concentrated and dialyzed against buffer containing 10 mM potassium phosphate, pH 7.4, and 20% (v/v) glycerol, in a Micro-ProDiCon concentrator (Pierce).

Analytical procedures. All spectral determinations were performed with an SLM Aminco DW2000 spectrophotometer. P-450 concentrations were determined according to the method of Omura and Sato (20). Protein concentrations were determined by the method of Lowry *et al.* (21), using bovine serum albumin as a standard. Discontinuous SDS-PAGE was carried out according to the method of Laemmli (22), and peptide mapping according to the method of Cleveland *et al.* (23), as described previously (24).

Immunochemical procedures. Polyclonal antibodies were raised against either rat hepatic P-450$_b$ (13–15) or the isozyme purified, as described above, from mice treated with CBD. New Zealand white rabbits (2.5 kg) were immunized subdermally with 100 μg of purified isozyme in complete Freund's adjuvant and were boosted with 50 μg of protein intramuscularly 3 weeks later. They were bled after an additional week and sera were obtained, aliquoted, and kept frozen at −70° until needed. Western blotting was performed as described previously (15), using 3 μg of microsomal protein. Slot-blotting was performed with a Minifold II slot-blot system (Schleicher and Schuell), essentially as for the Western blotting except that 0.3–0.6 μg of microsomal protein was used/slot.



CBD THC

Fig. 1. Chemical structures of CBD and THC.

Enzyme assays. Purified P-450 was reconstituted in the presence of saturating amounts of rat liver microsomal NADPH-P-450 reductase, dilauryl phosphatidylcholine (50 μg/ml), and NADPH (1 mM). Alkoxyresorufin *O*-dealkylase activities were determined as described (25, 26), except that the reaction was initiated by the addition of substrate (33 μM). Hexobarbital (27) and testosterone (15, 28) hydroxylase activities were assayed as previously described.

NH_2-terminal sequence analyses. Amino acid sequence determinations of the first 15 residues of the purified P-450 isozymes were performed by the Biomolecular Resource Center at the University of California, San Francisco. Samples containing 300–600 pmol of purified P-450 were subjected to automated Edman degradation, using an Applied Biosystems 470A gas-phase sequencer. The phenylthiohydantoin derivatives were identified and quantitated by reverse phase high pressure liquid chromatography, using an Applied Biosystems 120A liquid chromatograph.

Densitometry. Densitometric analyses were performed with a Hoefer GS 300 scanning densitometer in either transmittance (gels) or reflectance (blots) mode. Peak areas were quantitated by Gaussian fit integration, using the GS 370 densitometry program, and were found to be linear with respect to protein concentration.

Results

Isolation and Purification of P-450$_{CBD}$ from Mice

We have previously reported that an antibody raised against rat hepatic P-450$_b$ recognized a protein found in hepatic microsomes from mice that were repetitively treated with CBD but not found in hepatic microsomes from untreated mice (9). We, therefore, exploited this immunoreactivity as a "specific probe" for P-450$_{CBD}$ to aid in the development of the purification scheme. Microsomes from CBD-treated mice were solubilized and fractionated by anion-exchange chromatography. SDS-PAGE of the fractions revealed the presence of several major proteins in the molecular weight range of P-450 (M_r = 48,000–55,000), only one of which was found to be immunoreactive after Western blotting. Fractions that exhibited anti-P-450$_b$ immunoreactivity were pooled and subjected to additional chromatographic steps (as described in Experimental Procedures) until judged to be homogeneous by SDS-PAGE. The purified protein had a specific content of 10.6 nmol/mg and an apparent molecular weight of 51,600 (Fig. 2).

P-450$_{CBD}$

Spectral properties. P-450$_{CBD}$ exhibited an absolute oxidized absorption spectrum with maxima at 417, 534, and 568 nm, which shifted to 414 and 548 nm after reduction with dithionite (Fig. 3). Addition of CO after reduction shifted the maxima to 451 and 550 nm with a small shoulder at 420 nm, reflecting the presence of some P-420. Addition of metyrapone (1 mM) to reduced P-450 resulted in a ligand interaction similar to that previously shown to be specific for P-450$_b$ (14, 29). The reduced difference spectrum of the metyrapone-P-450 complex exhibited an absorbance maximum at 448 nm, with an extinc-

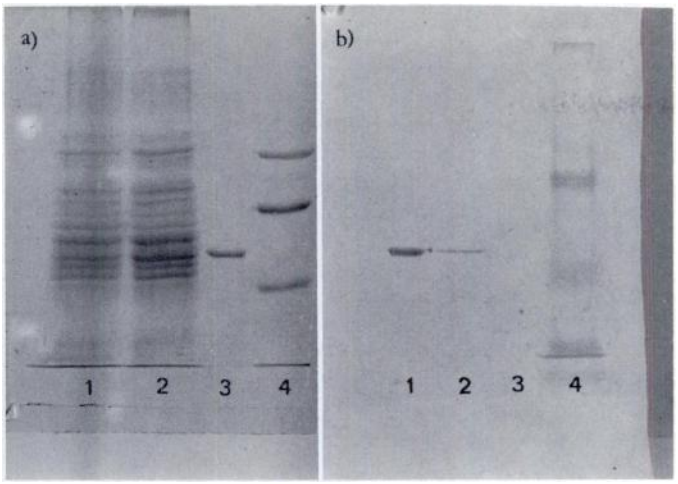


Fig. 2. SDS-PAGE and Western blots of hepatic microsomes and purified P-450$_{CBD}$. a, SDS-PAGE of hepatic microsomes from untreated (*lane 1*) or CBD-treated (*lane 2*) mice or P-450$_{CBD}$ (*lane 3*) purified from microsomes of CBD-pretreated mice. Protein standards (*lane 4*) of molecular mass 42.7, 66.2, and 97.4 kDa are given for comparison. b, Western blots of P-450$_{CBD}$ purified from CBD-treated mice (*lane 1*) and hepatic microsomes from CBD-treated (*lane 2*) or untreated (*lane 3*) mice. Prestained molecular mass protein standards (*lane 4*) of 17, 27, 39, 75, and 130 kDa are included for comparison.

tion coefficient of 43 ± 7 mM^{-1}cm^{-1}, similar to that reported for the purified rat liver isozyme (29).

Catalytic properties. When reconstituted with purified rat liver NADPH-P-450 reductase and lipid (see Experimental Procedures), P-450$_{CBD}$ catalyzed oxidative metabolism of several substrates at rates higher than those observed with hepatic microsomes from CBD-pretreated mice (Table 1). Inclusion of cytochrome b_5 had no significant effect on metabolic rates of any substrate studied (results not shown). Pentoxyresorufin, a substrate whose metabolism is below the limits of detection in microsomes from untreated mice but is induced >35-fold after CBD treatment (9), is found to be oxidized by P-450$_{CBD}$ at a rate greater than 4 nmol of resorufin formed/nmol of P-450/min (Table 1). This rate is approximately 10-fold higher than that observed with hepatic microsomes from CBD-treated mice. In addition, hexobarbital hydroxylation, a function markedly inhibited by acute CBD treatment but restored after repetitive CBD treatment in intact mice (9), is also found to be effectively catalyzed by P-450$_{CBD}$ at a rate 10-fold greater than that catalyzed by hepatic microsomes (Table 1). In order to further characterize P-450$_{CBD}$, testosterone hydroxylation was examined, because it has been used to characterize different hepatic P-450 isozymes in both rats (28, 30) and mice (31). P-450$_{CBD}$ exhibited high 16α-hydroxylase activity and appreciable 16β- and 6β-hydroxylase activity, as well as androstenedione-synthesizing capacity (Table 1).

NH_2-Terminal Sequence Analysis

NH_2-terminal sequence analysis of the first 15 amino acid residues (Table 2) suggests that this isozyme belongs to the PB-inducible gene family of isozymes designated IIB, according to the system of Nebert *et al.* (32). By this criterion, it is found to be highly homologous to rat liver P-450$_b$ [identical in 14 of the 15 amino acid residues identified (14)] as well as to rabbit liver LM$_2$ [10 of the 15 amino acid residues (33)].

Comparison with PB-Induced P-450 Isozyme from Mice

The CBD-induced P-450 isozyme appears to be very similar to PB-induced isozymes purified from rat (13, 14), rabbit (33), and mouse (31). It is immunoreactive with an antibody raised against rat P-450$_b$, interacts with metyrapone, *O*-dealkylates pentoxyresorufin with high specific activity, and has significant 16α- and 16β-testosterone hydroxylase activity, all well documented properties of the PB-inducible forms. In addition, it possess an NH_2-terminal sequence similar to those reported for PB-inducible isozymes purified from other species. Because of these many similarities, it was important to purify the PB-inducible form from mice in order to compare it with the CBD-inducible form. Utilizing the same purification procedure that

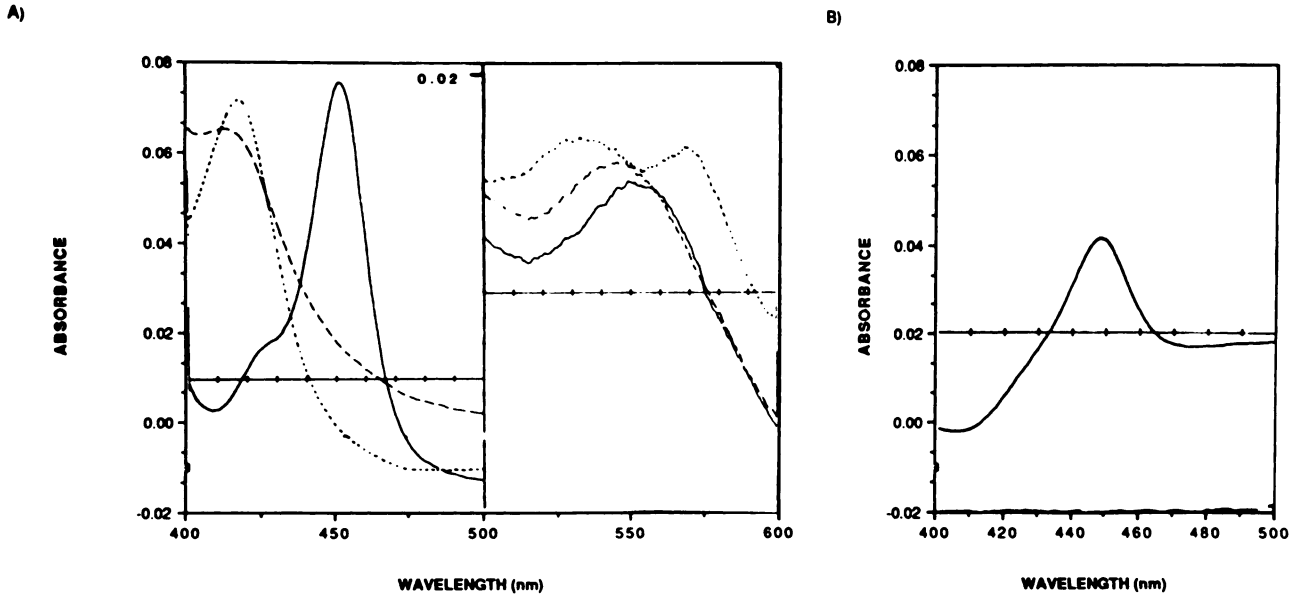


Fig. 3. Absorption spectra of CBD-inducible cytochrome P-450. A, Visible spectra (400–600 nm) were determined in 100 mM phosphate buffer, pH 7.4, containing 20% glycerol. Spectra shown are of purified P-450 that is oxidized (· · · ·), dithionite-reduced (– – –), or dithionite-reduced in the presence of CO (——). B, Difference spectrum of dithionite-reduced P-450 after the addition of metyrapone (1 mM) to the sample cuvette.

TABLE 1

Comparative catalytic activities of mouse hepatic P-450$_{CBD}$ and P-450$_{PB}$

Catalytic activities are reported as averages of the mean of at least three determinations. Purified P-450s (30–100 pmol) were reconstituted in the presence of saturating amounts of rat liver microsomal NADPH-P-450 reductase, dilauryl phosphatidylcholine (50 μg/ml), and NADPH (1 mM) in 0.1 M sodium phosphate buffer, pH 7.4. Substrate concentrations were 0.033, 1.0, and 0.28 mM for the alkoxyresorufins, hexobarbital, and testosterone, respectively.

Substrate	Product	Turnover	
		P-450$_{CBD}$	P-450$_{PB}$
		nmol of product/min/nmol of P-450	
Pentoxyresorufin	Resorufin	4.25	4.18
Benzyloxyresorufin	Resorufin	5.96	4.95
Ethoxyresorufin	Resorufin	0.76	0.62
Hexobarbital	3′-Hydroxyhexobarbital	23.2	17.7
Testosterone	16α-Hydroxytestosterone	6.62	5.83
	16β-Hydroxytestosterone	2.99	2.59
	6β-Hydroxytestosterone	0.78	0.70
	Androstenedione	2.26	2.12

was used for P-450$_{CBD}$, a P-450 was purified to homogeneity from hepatic microsomes from PB-pretreated mice (P-450$_{PB}$). This P-450 exhibited not only similar physical and functional characteristics but also a molecular weight identical to that of P-450$_{CBD}$. Its absolute (oxidized, reduced, and CO-reduced) spectra were identical to those previously described for P-450$_{CBD}$ (Fig. 3), as was the reduced difference spectrum of the metyrapone-P-450 complex, which yielded a similar extinction coefficient for the 448 nm complex (40 ± 8 mM^{-1}cm^{-1}). Furthermore, catalytic properties of P-450$_{PB}$ were not significantly different from those determined for P-450$_{CBD}$ (Table 1). NH_2-terminal sequence analysis of the first 15 amino acid residues revealed complete homology to the P-450$_{CBD}$ sequence (Table 2). Limited proteolysis of P-450$_{CBD}$ and P-450$_{PB}$ with either α-chymotrypsin or *Staphylococcus aureus* V8 protease generated peptide maps showing no appreciable differences between the two isozymes (Fig. 4).

Although CBD and PB treatment appear to induce similar P-450 isozymes, PB has been shown to induce microsomal PTR activity to a much greater extent than CBD (9). In order to determine whether this was due to the extent of induction of the enzyme, the microsomal content of the immunoreactive protein was quantitated by densitometric analysis. Hepatic microsomes from either untreated or CBD- or PB-treated mice were applied to a slot-blot apparatus and immunoblotted with an antibody raised against mouse hepatic P-450$_{CBD}$, and the blots were quantified by densitometric analysis (see Experimental Procedures). The amount of immunoreactive protein present was found to be 2- to 3-fold greater after PB treatment than after CBD treatment. This correlates extremely well with the corresponding PTR activity (correlation coefficient, r = 0.98), suggesting that PB is a quantitatively better inducer of the isozyme than CBD (Fig. 5).

Discussion

We have previously shown that acute CBD treatment of mice results in inactivation of hepatic microsomal P-450 and inhibition of drug metabolism, whereas repetitive CBD treatment resulted in the restoration of P-450 content and enzyme function (4, 5, 9) as well as in the stimulation of PTR activity (9). Concurrent with the stimulation of enzyme activity was the appearance of a protein that was not present in hepatic microsomes from untreated mice and that was immunoreactive with an antibody raised against P-450$_b$. We have now isolated and purified a P-450 isozyme from the livers of CBD-pretreated mice that we believe might be responsible for the observed anti-P-450$_b$ immunoreactivity and increased PTR activity. This CBD-inducible P-450 isozyme shares many physical and functional characteristics with the major PB-inducible P-450 isozymes purified from other species. The NH_2-terminal sequence of the first 15 amino acids of mouse P-450$_{CBD}$ is highly homologous to sequences previously reported for the PB-inducible P-450s from other species and it immunocross-reacts with antibodies raised against rat hepatic P-450$_b$. P-450$_{CBD}$ also possesses good catalytic activity with substrates such as pentoxy- and benzyloxyresorufin (26) and hexobarbital (34), which are known to be preferentially metabolized by rat hepatic P-450$_b$.

In addition, P-450$_{CBD}$ has a specific activity for testosterone 16α-hydroxylation comparable to that of a P-450 previously purified from hepatic microsomes from PB-treated mice, termed I-P-450$_{16\alpha}$ (31). However, in addition to 16α-hydroxytestosterone, I-P-450$_{16\alpha}$ was reported to produce only one other unidentified, less polar, testosterone metabolite, whereas P-450$_{CBD}$ generates additional testosterone metabolites oxidized at the 6β-, 16β-, and 17- positions. This discrepancy may be due to the thin layer chromatography system used to separate testosterone metabolites in the previous study, which may not have resolved the metabolites as well as the high pressure liquid chromatography system employed in our study. In fact, the unidentified metabolite appears to chromatograph similarly to 16β- and 6β-hydroxytestosterone (35). It is also possible that P-450$_{CBD}$ is different from I-P-450$_{16\alpha}$ or, conceivably, is contam-

TABLE 2

Comparison of NH_2-terminal sequences of mouse hepatic CBD- and PB-inducible P-450s with those of major PB-inducible P-450 isozymes from other species

The first 15 amino acid residues of mouse P-450$_{CBD}$ and P-450$_{PB}$ are shown. Only those residues of P-450$_b$ (14) and P-450 LM$_2$ (33) that differ from the residues identified for the mouse P-450$_{CBD}$ or P-450$_{PB}$ have been indicated.

P-450 isozyme	Residue 1 5 10 15
CBD-inducible mouse (P-450$_{CBD}$)	Met-Glu-Pro-Ser-Val-Leu-Leu-Leu-Leu-Ala-Leu-Leu-Val-Gly-Phe
PB-inducible mouse (P-450$_{PB}$)	Met-Glu-Pro-Ser-Val-Leu-Leu-Leu-Leu-Ala-Leu-Leu-Val-Gly-Phe
PB-inducible rat (P-450$_b$)	-Ile-
PB-inducible rabbit (P-450 LM$_2$)	-Phe- -Leu -Phe- -Ala- Leu-

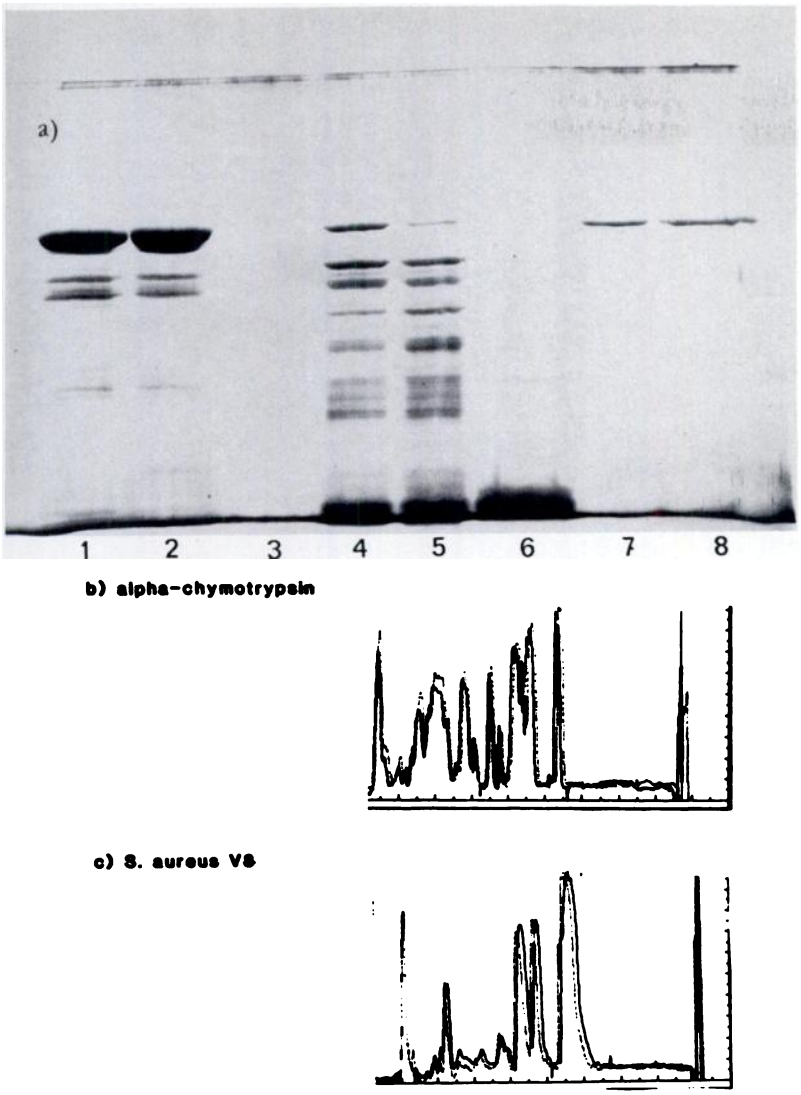


Fig. 4. Peptide maps of purified P-450$_{CBD}$ and P-450$_{PB}$. a, Purified P-450s were incubated in the presence or absence of α-chymotrypsin or *S. aureus* V8 protease for 3 min at 37°. *Lanes 1* and *2* contain peptides generated after incubation of *S. aureus* V8 protease with either P-450$_{PB}$ or P-450$_{CBD}$, respectively, while *lanes 4* and *5* contain peptides from comparable incubations with α-chymotrypsin. *Lanes 3* and *6* contain only the proteases, *S. aureus* V8 or α-chymotrypsin, respectively, while *lanes 7* and *8* contain P-450$_{PB}$ or P-450$_{CBD}$, respectively. b, Comparative densitometric scans of *lanes 4* and *5* (α-chymotryspin digests) of the gel shown in a. For clarity, scans are offset partially. c, Comparative densitometric scans of *lanes 1* and *2* (*S. aureus* V8 digests) of the gel shown in a.

inated with another P-450 isozyme that is inseparable by the purification techniques employed. On the other hand, in common with rat P-450$_b$ (14), mouse P-450$_{CBD}$ produces 16α- and 16β-hydroxytestosterone as well as androstenedione, reflecting a capability of this isozyme subfamily for multiple-site testosterone hydroxylations.

More recently (36), the NH_2-terminal amino acid sequence of I-P-450$_{16\alpha}$ was deduced from cDNA clones and revealed only limited homology to P-450$_{CBD}$. However, homology to P-450$_{CBD}$ was complete in the sequence deduced from another clone, which also had 95% sequence similarity to that of rat P-450$_b$ and P-450$_e$. Thus, although P-450$_{CBD}$ shares some similarities to I-P450$_{16\alpha}$, it more closely resembles the mouse orthologue of the major PB-inducible rat isozymes.

To further determine the extent to which CBD is a "PB-like" inducer of P-450, we purified the corresponding P-450 from mice treated with PB. This P-450 was indistinguishable from the CBD-induced P-450 by every criterion employed in this study: molecular weight, peptide mapping, immunoreactivity, NH_2-terminal sequence, visible absorption spectra, and catalytic function. Collectively, these data strongly suggest that CBD induces a P-450 isozyme in mouse liver that is similar or identical to that induced by PB. However, PB appears to induce this P-450 to a greater extent than CBD.

We have previously shown that, *in vitro*, hepatic microsomes from mice repetitively treated with CBD produce a nonpolar metabolite of CBD to a greater extent than do liver microsomes from either untreated or PB-treated mice (9). However, if PB and CBD induce the same isozyme and that isozyme were responsible for such metabolism, then one would expect to find greater production of the nonpolar CBD metabolite by liver microsomes from PB-treated mice, because PB is quantitatively a better inducer. Because this was not observed, a possible explanation for this discrepancy is that the nonpolar CBD metabolite is not produced by the PB- or CBD-inducible P-450. Indeed, in reconstitution experiments, neither P-450$_{CBD}$ or P-450$_{PB}$ produced any measurable metabolites of CBD, even in the presence of cytochrome b_5. Additionally, antibodies raised against purified P-450$_{CBD}$, failed to alter production of the nonpolar CBD metabolite in incubations of microsomes from CBD-pretreated mice. Thus, the increased production of the nonpolar metabolite by liver microsomes from CBD-pretreated mice might result from the induction of an additional P-450 isozyme (not yet purified) or, as suggested earlier, be due to

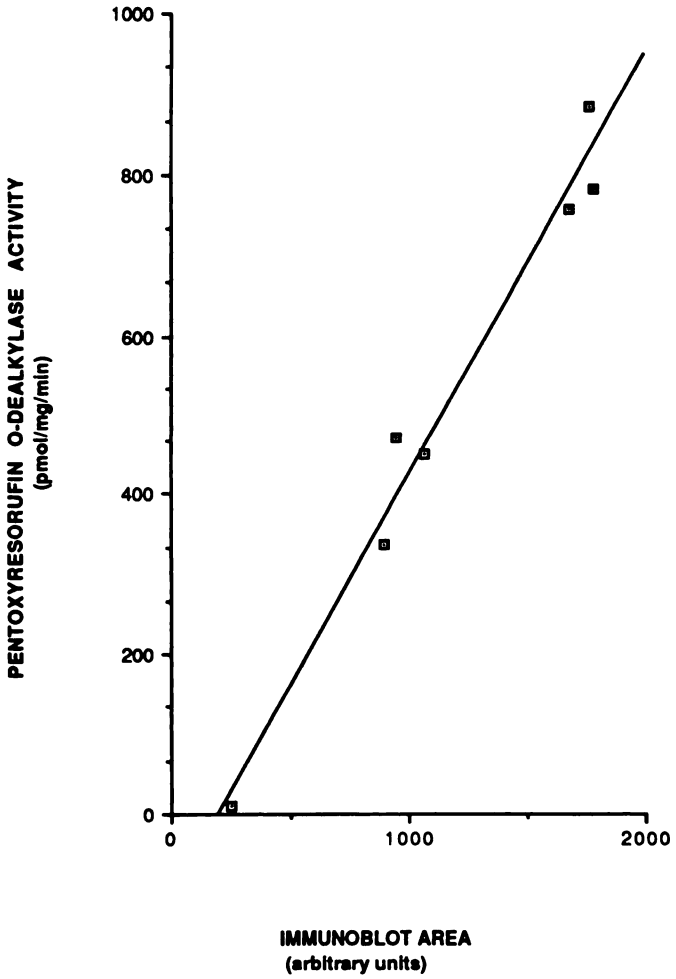


Fig. 5. Correlation of the immunoreactive protein content with PTR activity of hepatic microsomes. Hepatic microsomes (0.3–0.6 μg) from untreated or CBD- or PB-pretreated mice were applied to nitrocellulose membranes with a slot-blot apparatus and were probed with rabbit antibody raised against P-450$_{CBD}$. Blots were quantitated with a GS 300 scanning densitometer (Hoefer Scientific Instruments) using accompanying software (GS 370). The density of the blots was determined to be linear with respect to protein concentration for each of the samples.

CBD-mediated inactivation of a P-450 isozyme responsible for further oxidation of the metabolite in question (37, 38).

The observation that CBD is capable of hepatic P-450 induction is important for several reasons. CBD, when ingested therapeutically or illicitly as marijuana, may induce P-450 and influence the metabolism of other drugs taken concurrently, as suggested in several clinical studies (39–42). As an initial inactivator and subsequent inducer of P-450, it may also have a great effect on the physiological and psychological consequences of THC use, which may result in part from THC metabolites.

Elucidation of the effects of CBD on P-450 will allow for a better understanding of how CBD consumption may affect drug metabolism and help predict whether inhibition or induction of drug metabolism may result. Furthermore, because CBD appears to be a PB-like inducer of P-450, it may also increase the incidence of THC-induced teratogenesis, previously shown to be greatly enhanced by PB pretreatment (43).

In common with many P-450 inhibitors, CBD treatment initially results in P-450 inactivation, followed by a rebound P-450 induction after repeated treatment. Several rat hepatic P-450s have been shown to be either inhibited or induced by specific compounds, depending on the timing of administration. For instance, inhibition and rebound induction of hepatic P-450$_b$, P-450$_j$, and P-450$_p$ have been observed after treatment of rats with 2-isopropyl-4-pentenamide (44), pyrazole (45), or triacetyloleandomycin (46), respectively. Interestingly, CBD appears to be a unique inactivator/inducer, because it induces a P-450 isozyme that is not only different from the one it initially inactivated but is also resistant to further inactivation.

Because P-450 inactivation does not always result in rebound induction (47), it is possible that the persistence of the inhibitor, which is a function of its lipophilicity, may be important for its inductive potential, as suggested previously (48). THC, however, although similar in structure and lipophilicity to CBD (Fig. 1), does not induce P-450 or appreciably inhibit drug metabolism. Thus, it is tempting to speculate that CBD-mediated P-450 inactivation with consequently reduced hepatic CBD metabolism and elimination is responsible for the observed induction of P-450 by CBD.

In summary, we have isolated and purified a P-450 isozyme from hepatic microsomes of mice that were repetitively treated with CBD that is probably responsible for the altered microsomal drug-metabolizing activities observed after CBD treatment. This P-450 not only is indistinguishable from the major P-450 purified from hepatic microsomes of PB-pretreated mice but also is refractory to further inactivation by CBD. Such refractoriness may be due to the inability of this P-450 to metabolize CBD to a potentially reactive metabolite.

Acknowledgments

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