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Characterizations of Mouse Hepatic Microsomal Monooxygenase Catalyzing 11β-Hydroxylation of Osaterone Acetate

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ABSTRACT. Osaterone acetate (17α-acetoxy-6-chloro-2-oxa-4,6-pregnadiene-3,20-dione, OA) is a new steroidal antiandrogen. There is a marked species difference in the metabolism of OA in that 11β-hydroxylated metabolites are found in the plasma, feces, and urine of mice after oral administration of OA, but there is very little metabolism in rats and humans. OA reduces the adrenal gland weight in mice, but not in rats, and this effect in mice might be explained by the species difference in 11β-hydroxylation activity. The objectives of this study were to elucidate the enzyme(s) involved in this particular oxidation and to explain the species difference observed. Mouse hepatic microsomes oxidize OA to 11β-OH OA, and this oxidation requires NADPH as a cofactor. The use of various competitive and allosteric inhibitors of cytochrome P450 and flavin-containing monooxygenase (i.e. CO, *N*-octylamine, and methimazole) showed that the oxidation of OA was catalyzed by cytochrome P450. In microsomes from mice pretreated with phenobarbital (a CYP2B-selective inducer), 3-methylcholanthrene (a CYP1A-selective inducer), pregnenolone-16α-carbonitrile (a CYP3A-selective inducer), and EtOH (a CYP2E-selective inducer), an increase in the rates of oxidation was seen only in microsomes from EtOH-treated animals. However, metyrapone, a selective inhibitor for enzymes of the cytochrome P45011B and P4502B family, inhibited mouse hepatic microsomal 11β-hydroxylation by $< 30\%$. The results obtained showed that the production of 11β-OH OA may be catalyzed by a novel cytochrome P450 in mouse liver. BIOCHEM PHARMACOL **58**;2:335–341, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. 11β-hydroxylase; drug metabolism; mice; cytochrome P450; osaterone acetate; adrenal gland

OA§ is a new steroidal antiandrogen, and its metabolism has been investigated in several mammalian species [1]. Fifteen metabolites were isolated from animals and humans following oral administration, and there was a marked species difference in the metabolic pattern. The main metabolites were 17α-acetoxy-6-chloro-15β-hydroxy-2-oxa-4,6-pregnadiene-3,20-dione and 17α-acetoxy-6-chloro-21-hydroxy-2-oxa-4,6-pregnadiene-3,20-dione in rats and humans, respectively, whereas the main metabolite in mice was 17α-acetoxy-6-chloro-11β-hydroxy-2-oxa-4,6-pregnadiene-3,20-dione (11β-OH OA). There was no evidence of the existence of 11β-OH OA in humans.

Administration of OA reduces adrenal gland weight in mice, but not in rats [2], and this effect in mice might be explained by a species difference of 11β-hydroxylation activity, because 11β-OH OA exhibits higher binding affinity for corticoid receptors than the parent compound [3].

In quantitative terms, the liver is the most important site of hydroxylation for the majority of compounds. Although the overall quantitative contribution of extrahepatic hydroxylation is generally lower than that of the liver, extrahepatic metabolism can play a specific biologic role. The adrenal glands have been shown to contain specific cytochrome P450 isoforms, and these enzymes are responsible for the 11β-hydroxylation of endogenous steroids. Until recently, the kidney received relatively little attention as far as drug metabolism was concerned. However, all drugs that are excreted in the urine must pass through the kidney, and this organ contributes to the total metabolism of many drugs in the body. Although the presence of a Phase I detoxication system in the kidney has been established, there is not much information about its significance [4, 5].

The objective of this study was to clarify the enzymes responsible for the production of 11β-OH OA, and it involved the determination of the adrenal, hepatic, and renal contributions to the 11β-hydroxylation of OA. In addition, the enzyme(s) catalyzing the 11β-hydroxylation of OA was characterized.

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§ *Abbreviations*: OA, osaterone acetate; FMO, flavin-containing monooxygenase; PB, phenobarbital; 3-MC, 3-methylcholanthrene; and PCN, pregnenolone-16α-carbonitrile.

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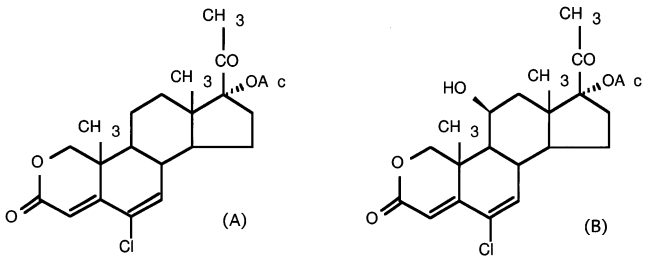

FIG. 1. Chemical structures of OA (A) and 11β-OH OA (B).

MATERIALS AND METHODS

Materials

[17α-*Acetate*-^{14}C]OA ([^{14}C]OA) was synthesized from [^{14}C]Ac_2O by the Daiichi Pure Chemical Co., Ltd. OA, 11β-OH OA, 17α-propanoyl OA, and [$^{18}O_2$, ^{2}H]OA (Fig. 1) were supplied by the Teikoku Hormone Mfg. Co. Ltd. [$^{18}O_2$, ^{2}H]15β-OH OA was isolated from rat bile following oral administration of [$^{18}O_2$, ^{2}H]OA. NADPH and NAD^+ were purchased from the Kohjin Co. Ltd. $NADP^+$ was obtained from the Oriental Yeast Co. Ltd. Glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from the Sigma Chemical Co. All other chemicals were of analytical reagent grade.

Animals

Ten-week-old male ddY mice were purchased from Japan SLC Inc., and were given tap water and MMC diet (Funabashi Farm) *ad lib.* for more than 1 week.

Tissue Localization of OA 11β-Hydroxylase Activity

Mice were killed by decapitation. The adrenal glands, livers, and kidneys were removed immediately, and the tissues were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4). Inactivation was performed by heating at 90° for 3 min. The enzyme assay mixture contained NADPH-generating system (0.25 μM NADPH, 15 μM glucose-6-phosphate, 4 U glucose-6-phosphate dehydrogenase, and 5 μM $MgCl_2$), phosphate buffer (0.1 M, pH 7.4), each homogenate (18.8 to 125 mg eq. of wet tissue/mL), and [^{14}C]OA (8 μg/18.5 kBq) in a final volume of 2 mL. The reaction was started by adding [^{14}C]OA. The mixture was incubated at 37°, with shaking, for 60 or 120 min. The reaction mixture was then placed on ice and acidified with 1.5 mL of 0.1 M HCl. The mixture was centrifuged at 3000 *g* for 10 min, and the supernatant was dried under reduced pressure, reconstituted in chloroform, and spotted onto a silica-gel TLC plate (Kieselgel $60F_{254}$ 0.25-mm thickness, Merck). The solvent system was chloroform:acetone (20:1, v/v). The TLC plate was placed in contact with X-ray film (Kodak X-ray film) for 3 weeks to detect the radioactivity. Silica gel corresponding to the 11β-OH OA and 11β,15β-$(OH)_2$ OA bands was removed, eluted with MeOH, and evaporated to dryness. The isolated metabolites were examined by LC-MS. LC-MS was performed using an M-2000 (Hitachi) equipped with an SPD-6A UV detector (Shimadzu) and a Capcell Pak C_{18} column (4.6 × 250 mm, SG-120). The mobile phase was 65% MeOH at a flow rate of 1.0 mL/min. The detection was performed at *m/z* 423 and total ion (*m/z* 30–500). The UV intensity was monitored at 270 nm.

Preparation of Subcellular Fractions

The hepatic nuclear, mitochondrial, cytosolic, and microsomal fractions were prepared as described by Guengerich [6]. In this method, the untreated mice were killed by decapitation and their livers were removed immediately. The tissues were homogenized in ice-cold 0.1 M Tris–HCl buffer (pH 7.4), containing 0.1 M KCl, 1 mM EDTA, and 20 μM dibutylhydroxytoluene (BHT). The homogenates were centrifuged at 900 *g* for 5 min, and the nuclear precipitates were obtained. The supernatants were subsequently centrifuged at 9000 *g* for 20 min, and the mitochondrial fraction was obtained. The resulting supernatants were centrifuged at 105,000 *g* for 60 min, and the microsomal pellets and cytosolic fractions were separated. Microsomal pellets were resuspended in Tris–HCl buffer (pH 7.4), containing 0.1 M KCl, 1 mM EDTA, and 20 μM BHT, and centrifuged at 105,000 *g* for 60 min.

Subcellular Distribution of 11β-Hydroxylase

The enzyme assay mixture contained NADPH-generating system (0.5 mM $NADP^+$, 5 mM glucose-6-phosphate, 5 mM $MgCl_2$, and 2 U glucose-6-phosphate dehydrogenase), Tris–HCl buffer (50 mM, pH 7.4), each fractional protein (700–4150 μg/mL) and substrate (10 μM) in a final volume of 1 mL. The reaction was started by adding OA. The mixture was incubated at 37°, with shaking, for 10 min. Then the reaction mixture was placed on ice and acidified with 1 mL of 2 M HCl. After the addition of 50 ng of 17α-propanoyl OA as an internal standard, the mixture was applied to a Bond Elut C18 cartridge (3 mL column volume) that had been rinsed with 2 vol. each of MeOH and distilled water. The cartridge was washed with 4 mL of distilled water and 2.5 mL of 30% (v/v) acetonitrile. Then the metabolites were eluted with 3 mL of 60% (v/v) acetonitrile, and the eluate was evaporated *in vacuo*. The residue was dissolved in 100 μL MeOH and subjected to HPLC. HPLC was performed using a 600E multi-solvent delivery system (Waters) equipped with an SPD-6A UV detector (Shimadzu) and a YMC-Pak AQ-312 column (6 × 150 mm, Yamamura). The mobile phase was MeOH: acetonitrile:water (24:36:40, by vol.) at a flow rate of 1.0 mL/min and a column temperature of 40°. The detection was performed at a wavelength of 275 nm. The retention times of 17α-propanoyl OA, OA, and 11β-OH OA were 33.0, 21.9, and 17.1 min, respectively. The peak-area ratio of 11β-OH OA to 17α-propanoyl OA was measured.

Kinetic Studies

One-milliliter aliquots containing 12.5 to 100 mg equivalent of wet tissue were incubated at 37° with various concentrations of OA (0.6 to 10 μM). Under these conditions, 11β-hydroxylase activity is linear with time for at least 15 min at 37° at protein concentrations up to 50 mg equivalent of wet tissue. V_{max} and K_m were determined from Lineweaver–Burk plots.

Inhibition Studies

Incubation mixtures contained 50 mg microsomal protein, 50 mM Tris–HCl (pH 7.4), and an NADPH-generating system and substrate (10 μM) in a final volume of 1 mL. Four compounds (*N*-octylamine, α-naphthoflavone, metyrapone, and methimazole) were tested for their inhibitory effects on OA 11β-hydroxylation. The inhibitory effect of each chemical was determined at three concentrations (0.1, 0.5, and 1.0 mM). These inhibitors were added in Tris–HCl buffer (pH 7.4). For the experiment using CO, the reaction mixture was the same as above. Before addition to the incubation tubes, the protein component was gassed with a direct flow of CO gas. Reaction mixtures containing inhibitors were incubated at 37°, with shaking, for 10 min, and the 11β-hydroxylation reaction was initiated by adding OA. The reaction was terminated, and 11β-hydroxylase activity was determined using HPLC as described above. 11β-Hydroxylation rates in the presence of inhibitor were compared with appropriate controls, and the results were expressed as a percentage.

Induction Studies

Mice were separated into their respective treatment groups. PB-treated mice drank water containing 0.5 mg/mL of PB for 10 days. 3-MC-treated mice were injected i.p. with 50 mL/kg body weight of 0.1 mg/mL of 3-MC in corn oil [7], and at 120 hr after administration the mice were killed. PCN-treated mice were given by daily i.p. injection 50 mL/kg body weight of 0.1 mg/mL of PCN in corn oil for 5 days [8], and were killed 24 hr after the final administration. Ethanol (EtOH)-treated mice drank 30% (v/v) EtOH/30% (w/v) sucrose solution for 3 weeks [9]. Untreated mice were divided into two groups: one group was given daily i.p. injections of 50 mL corn oil/kg body weight for 5 days, while the other group was given the normal diet and water. Microsomal fractions were prepared, and the 11β-hydroxylation activity was assayed by HPLC. The method used was described above.

General Procedures

Protein concentration was determined by the method of Lowry *et al.* [10] using BSA as the standard. Ethoxycoumarin-*O*-deethylase activity was determined by the method of Aitio [11]. The statistical significance of any differences was calculated using Student's *t*-test.

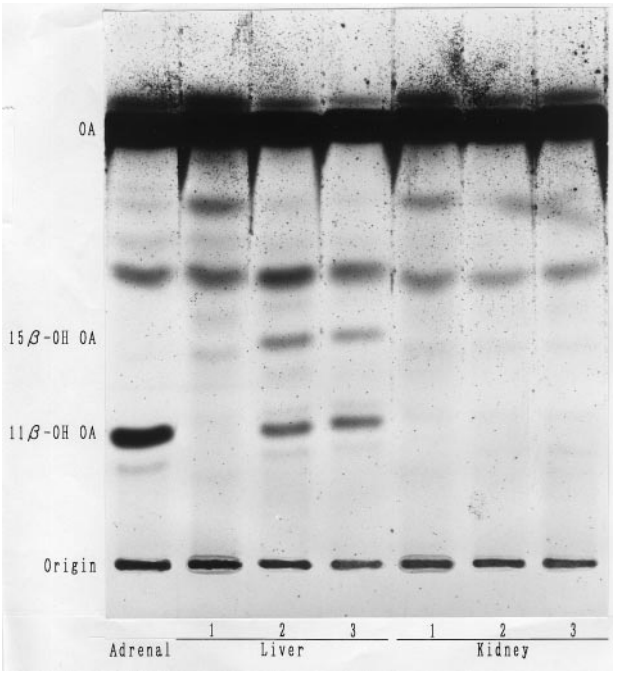

FIG. 2. Thin-layer chromatogram of OA metabolites from adrenal, liver, and kidney homogenates after incubation with [^{14}C]OA. OA was incubated with mice adrenal, liver, and kidney homogenates. The metabolites were analyzed by TLC. Key: (1) Heat inactivation, incubation for 60 min. (2) Incubation for 60 min. (3) Incubation for 120 min.

RESULTS

Tissue Localization of OA 11β-Hydroxylase Activity

Figure 2 shows a typical TLC profile after incubating [^{14}C]OA with adrenal gland, hepatic, and renal homogenates of mice. The spot corresponded to that of 11β-OH OA, which was found in adrenal gland and liver, but not in kidney. The adrenal glands have been shown to contain specific cytochrome P450 isoforms responsible for the 11β-hydroxylation of endogenous steroids. On the other hand, there have been no reports indicating that extraadrenal cytochrome P450s catalyze the 11β-hydroxylation of steroids. To discriminate between a true enzymatic product and an artificial product, heat-inactivation experiments were performed in liver. No 11β-OH OA was produced by the heat-inactivation treatment. The corresponding spot was scraped off the TLC plate, and the metabolite was isolated and analyzed by LC-MS. The retention time and fragment ions were in good agreement with those of the synthetic sample of 11β-OH OA (Fig. 3). Activity was seen in the hepatic homogenates. OA was transformed to 11β-OH OA by mouse hepatic homogenates.

Characterizations of Hepatic 11β-Hydroxylase

In the presence of NADPH, subcellular localization of OA 11β-hydroxylation activity was examined and compared with that of the microsomal fraction. The specific activity of the various fractions is shown in Table 1. The highest activity was seen in the microsomal fraction. OA was

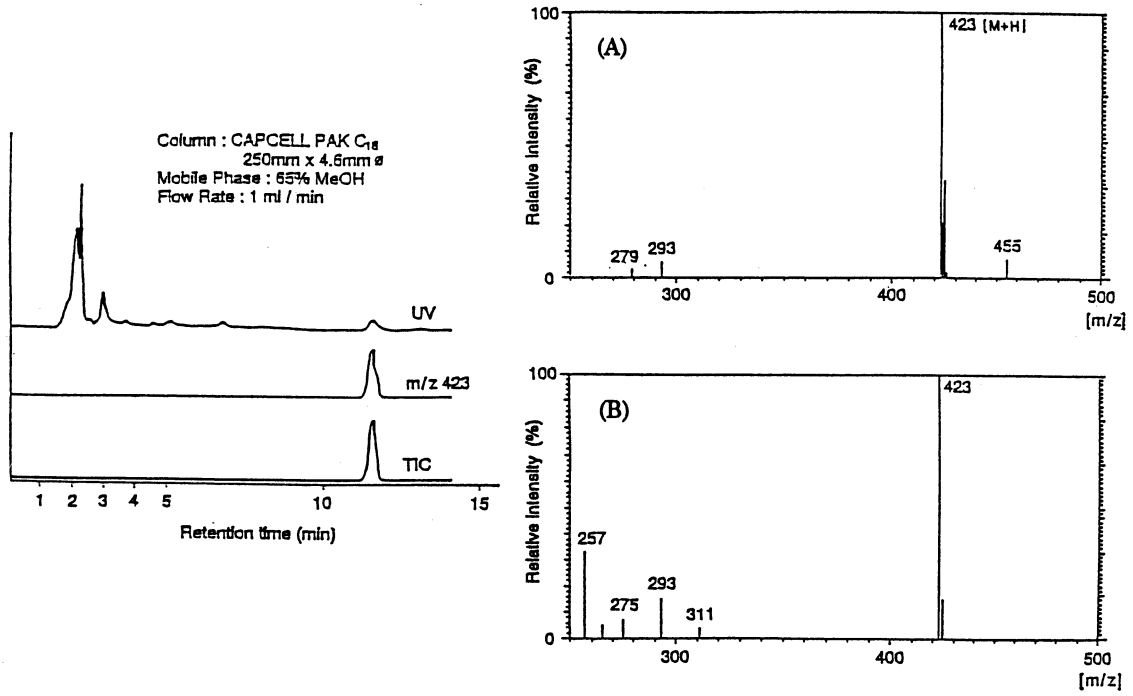

FIG. 3. Mass spectra of 11β-OH OA formed from OA by mice hepatic homogenate. OA was incubated with mouse liver homogenate, and the 11β-OH OA formed was isolated. The metabolite was analyzed by LC-MS. TIC (total ion chromatogram) was performed by detection at *m/z* 30–500. (A) Authentic sample; and (B) metabolite.

preferentially transformed to 11β-OH OA in mouse hepatic microsomes.

The reaction required NADPH for maximal activity (Table 2). NADH was much less effective as the cofactor and actually showed an inhibitory effect when added together with NADPH. $NADP^+$ and NAD^+ were ineffective as the cofactor. Hepatic microsomes of mice catalyzed the oxidation of OA to 11β-OH OA in the presence of an NADPH-generating system.

Kinetic Study

Hepatic microsomal formation of 11β-OH OA gave linear plots with protein concentrations up to 50 mg equivalent of wet tissue/mL. Also, the enzyme activity gradually increased with substrate concentration up to 10 nM. The reaction was linear until 15 min of incubation time. As shown in Fig. 4, the Lineweaver–Burk plot was a single line.

TABLE 1. Subcellular localization of NADPH-dependent 11β-OH OA forming activity in mouse liver

Subcellular fraction	11β-OH OA formed (pmol/min/mg protein)	Percent of microsomes
Nuclei	ND	
Mitochondria	0.587 ± 0.092	7
9000 *g* Supernatant	1.676 ± 0.049	19
Microsomes	8.855 ± 0.866	100
Cytosol	0.609 ± 0.183	7

Data are expressed as means ± SD of four experiments. ND = not detected.

The V_{max} and K_m values were 0.205 pmol/min/g of liver and 3.534 μM, respectively.

Inhibition Studies

Hydroxylation in liver has been demonstrated to be catalyzed by FMO and cytochrome P450. Hepatic microsomal formation of 11β-OH OA was inhibited by the addition of *N*-octylamine (0.1 mM) and CO gas to the incubation mixture, the degree of inhibition being 44% and more of the control, respectively (Table 3). These compounds are known to be typical inhibitors of cytochrome P450-dependent reactions. Metyrapone, an inhibitor of PB-inducible isozymes [8] and a typical inhibitor of 11β-hydroxylase in the adrenal gland, inhibited the reaction, whereas α-naphthoflavone, a selective inhibitor of CYP1A1, and methimazole, a typical inhibitor of FMO, did not.

TABLE 2. Cofactor requirement for 11β-OH OA formation with mouse hepatic microsomes

Cofactors	11β-OH-OA formed (pmol/min/mg protein)	Percent of NADPH
None	0.803 ± 0.191	9
NAD+	0.947 ± 0.003	11
NADH	2.796 ± 0.226	33
NADP+	0.824 ± 0.102	10
NADPH	8.505 ± 0.182	100
NADPH + NADH	7.960 ± 0.260	94

Data are expressed as means ± SD of three experiments. The concentration of each cofactor added to the incubation mixture was 1 mM.

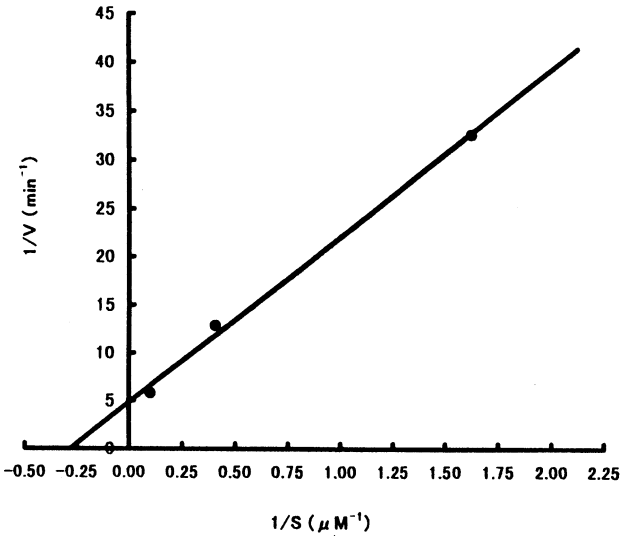

FIG. 4. Lineweaver–Burk plot of OA 11β-hydroxylase activity. The line was derived from least-squares fit to data points (r^2 = 0.955). The apparent K_m and V_{max} values were obtained from these data.

Induction Studies

To clarify which mouse isoform contributes to this oxidation system, incubations were performed in the presence of microsomes treated with several inducers (Table 4). Pretreatment with EtOH, an inducer of CYP2E1, increased significantly the hepatic microsomal formation of 11β-OH OA on the basis of microsomal protein. PB, an inducer of CYP2B, did not affect the microsomal formation of 11β-OH OA. PCN, an inducer of CYP3A2, and 3-MC, an inducer of CYP1A, did not affect the formation. On the other hand, *O*-deethylation of 7-ethoxycoumarin was affected only by PB.

TABLE 3. Effects of various inhibitors on 11β-OH OA formation with mice hepatic microsomes

Inhibitors	Concentration (mM)	11β-OH OA formed (pmol/min/mg protein)	Percent of control
Experiment 1			
Control		4.342 ± 0.740	100
N-Octylamine	0.1	2.443 ± 0.460	56
	0.5	ND	ND
CO		ND	ND
Experiment 2			
Control		5.730 ± 0.481	100
α-Naphthoflavone	0.1	6.347 ± 1.082	111
	0.5	5.631 ± 0.639	98
	1.0	5.787 ± 0.789	101
Metyrapone	0.1	2.401 ± 0.238	42
	0.5	1.530 ± 0.022	27
	1.0	1.321 ± 0.088	23
Methimazole	0.1	6.171 ± 0.859	108
	0.5	4.052 ± 0.247	71
	1.0	4.678 ± 0.756	82

Data are expressed as means ± SD of three experiments. ND = not detected.

TABLE 4. Effects of pretreatment with inducers on 11β-OH OA formation with mouse hepatic microsomes

Treatment	Activity: OA 11β-hydroxylase (pmol/min/mg protein)	Activity: 7-Ethoxycoumarin-O-deethylase (nmol/min/mg protein)
Experiment 1		
Control	5.771 ± 1.446	1.188 ± 0.181
3-MC	5.488 ± 1.084	1.171 ± 0.213
PCN	4.335 ± 0.569	1.266 ± 0.191
Experiment 2		
Control	9.11 ± 2.533	1.419 ± 0.208
PB	6.64 ± 1.173	6.972 ± 0.431*
EtOH	15.57 ± 2.640†	1.674 ± 0.368

Data are expressed as means ± SD of five animals.
*,†Significant difference vs control: *$P < 0.0001$, and †$P < 0.001$.

DISCUSSION

In a previous study, we have shown a species difference in the metabolism of OA [1]. 11β-Hydroxylated metabolites were found in the plasma, feces, and urine in mice, but there was little evidence of these in human plasma. 11β-Hydroxylation of exogenous steroids represents a novel biotransformation pathway. Steroid hormone biosynthesis requires sequential action of a related group of cytochrome P450 enzymes. The site of the 11β-hydroxylation of steroids is localized mainly in the adrenal cortex mitochondria. The major steroid products of the adrenal cortex are aldosterone, the primary mineralocorticoid, and corticosterone, the predominant glucocorticoid. These two steroid classes are produced in different cortical compartments: the outer zona glomerulosa produces mineralocorticoids, and the inner zonae fasciculata/reticularis produce glucocorticoids. The production of aldosterone by the zona glomerulosa is under dual control by the renin–angiotensin system [12] and extracellular potassium, whereas ACTH largely determines the rate of glucocorticoid production by the inner cortical zones. In rats [13, 14] and humans [15–19], two distinct 11β-hydroxylase proteins are expressed in adrenal cortex. In mice, this selective expression of 11β-hydroxylase isozymes in different adrenocortical zones very likely contributes significantly to the functional separation into glucocorticoid- and mineralocorticoid-producing compartments [20].

Biologic hydroxylation of drugs usually is catalyzed by cytochrome P450s, which are mainly localized in liver and kidney. The present study was designed to characterize the site of the enzymatic activity involved in OA 11β-hydroxylation. As shown in Fig. 2, this activity was detected in hepatic and adrenal homogenates, but not in renal homogenate. It is also possible that the liver serves to produce 11β-OH OA in mice. 11β-Hydroxylase activity toward steroids has never been reported in the liver before. Adrenocortical mitochondrial cytochrome P450 isozymes, such as the CYP11 family, normally synthesize steroids with a very strict substrate specificity, and adrenal P450s gener-

ally are not concerned with xenobiotic metabolism. However, P450c11, the adrenocortical mitochondrial cytochrome P450 isozyme, additionally has been shown to metabolize and bioactivate the adrenotoxic environmental pollutant 3-methylsulfonyl-2,2-bis(4-chlorophenyl)-1,1-dichloroethane [21].

OA 11β-hydroxylase activity was found in the hepatic microsomal fraction. This subcellular localization was in agreement with that of general cytochrome P450s (Table 1). Morohashi *et al.* [22] have shown the characteristic sequence between microsomal and mitochondrial type P450s. This has led to the view that distinct 11β-hydroxylase proteins are expressed in mouse liver. The next study was designed to characterize the cofactor requirement. NADPH was an obligatory cofactor for this reaction (Table 2). However, this result suggests that cytochrome b_5 has no role in the oxidation of OA because NADH did not affect the reaction [23]. The stimulatory actions of b_5 on testosterone 6β-hydroxylation by CYP3A4 and 17,20-lyase activity by P450c17 exhibit an allosteric effect [24]. This cofactor requirement and the subcellular localization were in agreement with those of general cytochrome P450s.

To discriminate between FMO and cytochrome P450 as catalysts of OA 11β-hydroxylation, positive effectors of FMO [25] and inhibitors for cytochrome P450 [26] were used. The observations that this enzymatic activity is in the microsomal fraction, that it requires NADPH, and that there is complete inhibition of hydroxylation by the cytochrome P450 inhibitor CO strongly suggest that this hydroxylation is catalyzed by cytochrome P450 enzymes. This finding was further supported by investigating the influence of *N*-octylamine and methimazole on FMO. Methimazole, an inhibitor of FMO, had no effects. The absence of FMO in the hydroxylation of OA was finally supported by the effects of *N*-octylamine, a known cytochrome P450 inhibitor and an FMO positive effector. By using cytochrome P450 inhibitors, the contribution by enzymes of the cytochrome P450 family to this oxidation reaction was demonstrated. To further elucidate the nature of the enzyme, we used selective inhibitors of cytochrome P450 isoforms. α-Naphthoflavone, a selective inhibitor of CYP1A1/2, had no effect on the activity, but it was inhibited by metyrapone. It is well known that metyrapone inhibits 11β-hydroxylase in the adrenal gland. This finding suggests that this 11β-hydroxylase has a stereochemical configuration similar to that of the 11β-hydroxylase in the adrenal gland. In liver, metyrapone inhibits the metabolism of a variety of substrates in microsomes from PB-treated rats but has little influence on metabolism by microsomes from 3-MC- and EtOH-treated rats [8].

Pretreatment with EtOH increased significantly the formation of 11β-OH OA. EtOH induces the metabolism by CYP2E1. A part of the induction mechanism is protein stabilization [27]. On the other hand, the induction mechanism of dexamethasone, another CYP2E1 inducer, is unknown. The metabolism of 7-ethoxycoumarin involves cytochrome P450 mediated oxidative *O*-deethylation. 7-Ethoxycoumarin has been used widely as an indicator of metabolite viability in hepatocytes and subcellular fractions [28, 29]. Treatment with PB, an inducer of the CYP2B family, induced the cytochrome P450s responsible for the deethylation of 7-ethoxycoumarin to 7-hydroxycoumarin, but did not induce the specific form of cytochrome P450 responsible for OA 11β-hydroxylation. CYP3A and CYP1A1/2 have been described as being isoforms for many steroids. However, treatment with PCN and 3-MC, inducers of CYP3A and CYP1A1/2, respectively, did not induce either of these reactions. Metabolism by CYP2E1, an enzyme with substrate homeostatic regulation via ACTH, is not inhibited by metyrapone. An enzyme with substrate specificity and immunochemical characteristics similar to rat liver cytochrome P4502E1 has been detected in the kidney of the male mouse [30]. In the kidney microsomes from this male mouse, the rates of oxidation of chloromethane and chlorzoxazone, specific substrates for cytochrome P4502E1, were only half that in liver microsomes [31]. These results do not agree with those obtained by the inhibitors and the subcellular distribution.

It is also important to note that 11β-OH OA is produced extra-adrenally. If 11β-OH OA is produced only in the adrenal gland, the reduction of the adrenal gland may be prevented by homeostatic regulation via ACTH. The mouse hepatic microsomal cytochrome P450 catalyzes 11β-hydroxylation of OA, and this enzyme would explain part of the reduction of adrenal gland weight in mice. Moreover, oxidoreduction at carbon 11 of corticoids, carried out by 11β-hydroxysteroid dehydrogenases, modulates the levels of biologically active corticoids. Further work is needed to establish the mechanism behind the species difference in the reduction of adrenal gland.

In conclusion, our findings indicate that mouse extra-adrenal enzymes catalyze the 11β-hydroxylation of OA. Furthermore, our data indicate that a mouse hepatic microsomal cytochrome P450 enzyme catalyzes the 11β-hydroxylation of OA, and hepatic FMO appears to play no role. In the past decade, many investigations have been performed looking at the multiple forms of P450 in laboratory animals and humans. However, the results presented in our study provide the first example of the 11β-hydroxylation of exogenous steroids. In addition, EtOH, a selective inducer of CYP2E1, increases the activity. There was no isoform with an inhibiting or inducing effect on this 11β-hydroxylation. The mouse hepatic microsomes catalyzed this oxidation of OA to 11β-OH OA, which may be catalyzed by a novel cytochrome P450.

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