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Major Differences in the Specificity and Regulation of Mouse Renal Cytochrome P-450-Dependent Monooxygenases

A Comparison of Xenobiotic and Endogenous Substrates

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SUMMARY

In the present study we have investigated the influence of sex on the specificity of mouse renal microsomes toward endogenous and xenobiotic substrates. Renal microsomes from C3H/HeJ mice were characterized by the following: a 4- to 5-fold male predominance in cytochrome P-450 concentration; a difference between male and female renal microsomes in the absorption maximum for the reduced P-450·CO complex, 450 and 452 nm, respectively; a lack of a sex difference in lauric acid 12-hydroxylase activity; an 18-fold sex difference (M > F) in progesterone 16α-hydroxylase activity; and 8- to 10-fold sex differences (M > F) in progesterone 15α-hydroxylase, dimethylnitrosamine demethylase, and lauric acid 11-hydroxylase activities. Treatment of female mice with testosterone propionate selectively induced lauric acid 11-hydroxylase and dimethylnitrosamine demethylase activities 8- and 14-fold, respectively, but had no effect on progesterone 15α- and 16α-hydroxylase activities or on the high female rate of lauric acid 12-hydroxylation. Inhibition studies conducted with male mouse renal microsomes revealed that of the substrates examined, only testosterone inhibited the 15α- and 16α-hydroxylations of progesterone *in vitro*. In addition, progesterone 15α-hydroxylase was distinguished from 16α-hydroxylase by the greater degree of testosterone inhibition (68 and 44%, respectively) and by sensitivity to metyrapone inhibition. Mouse renal cytochrome P-450 heterogeneity is indicated by the selective effects of androgen induction and metyrapone inhibition. Moreover, distinct modes of regulation are observed between the isozymes involved in steroid hydroxylation and those which catalyze the 11- and 12-hydroxylations of lauric acid in mouse renal microsomes.

INTRODUCTION

The cytochrome P-450-dependent monooxygenase system is involved in the oxidation of a wide array of xenobiotic and endogenous compounds (1). It is now well established that the broad substrate specificity of the hepatic monooxygenase system reflects the existence of multiple forms of the cytochrome P-450 hemoprotein which is the terminal electron acceptor and substrate-binding moiety of this enzyme complex. The various isozymes of cytochrome P-450 possess different but sometimes overlapping substrate specificities and can vary independently as a function of age, sex species, strain, and exposure to xenobiotics (2–6).

Although the hepatic cytochrome P-450 monooxygenase system of many species has been well characterized, surprisingly little is known about the renal cytochrome P-450-dependent monooxygenase system (7). Many characteristics of the rat renal monooxygenase system differ from those of the rat hepatic monooxygenase system. Some examples of the differences between the two monooxygenase systems are a lower concentration of cytochrome P-450 in renal microsomes resulting in generally lower rates of xenobiotic metabolism (8), renal refractoriness to phenobarbital induction (9), absence of sex differences in renal cytochrome P-450 and associated monooxygenase activities (10, 11), and different substrate specificities between liver and kidney toward endogenous compounds such as steroids and fatty acids (12, 13).

To obtain a better perspective on the metabolic role of the kidney in certain toxicologic and carcinogenic processes, further characterization of the renal monooxygenase system is required. While recent attention has focused primarily on the characterization and localization of cytochrome P-450 isozymes in porcine and rabbit kidney (14, 15), much less is known about the renal monooxygenase systems of other species. This is especially apparent in the mouse, a species which offers the advantages of genomic homogeneity. Recently, large sex differences have been reported in the cytochrome P-450

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concentration of mouse kidney microsomes and in the hydroxylation of testosterone by kidney microsomes (16). Interestingly, in this previous study, the low levels of female renal testosterone hydroxylase activity remained refractory to induction by testosterone propionate administration in spite of large increases in renal cytochrome P-450 concentration. The objective of the present study was to examine the substrate specificity and regulation of the low levels of cytochrome P-450 in female renal microsomes with regard to both endogenous and xenobiotic substrates. Testosterone and 7-ethoxycoumarin are model monooxygenase substrates which have been useful in characterizing multiple forms of hepatic cytochrome P-450. Progesterone was also selected as a substrate to test whether the same constraints placed on female testosterone metabolism, with regard to androgen introduction, also applied to a C21 steroid substrate. Lauric acid represents another class of endogenous substrates, the fatty acids, which are known as model substrates for the renal monooxygenase system (12–14). Finally, dimethylnitrosamine was selected as a substrate because of the well established sex differences found in the metabolic activation of dimethylnitrosamine to mutagens in microbial test systems employing mouse renal S9 fractions in the activation mixture (17). We have selected the C3H/HeJ strain for our studies because recent investigations have revealed that the female renal microsomes of this strain have a higher cytochrome P-450 concentration and higher steroid hydroxylase activity than other strains examined. Comparison of the various enzyme activities with regard to regulation by sex and androgen induction should provide insight into the substrate specificity and multiplicity of the mouse renal cytochrome P-450 system.

MATERIALS AND METHODS

Chemicals. [4-^{14}C]Testosterone (56.1 mCi/mmol) and *N*-[*methyl*-^{14}C]nitrosodimethylamine (54.0 mCi/mmol) were purchased from New England Nuclear. Prior to use, [^{14}C]dimethylnitrosamine was purified by Dowex-1-bisulfite chromatography as described by Den Engelse *et al.* (18). [1-^{14}C]Lauric acid (36.3 mCi/mmol) was obtained from Amersham Corp.

Progesterone, which was purified by recrystallization twice from ethanol/water was purchased from Sigma. 6α-, 6β-, 16α-, and 21-Hydroxyprogesterone were purchased from Steraloids, Inc., Wilton, NH. 15α-Hydroxyprogesterone was a gift from Dr. A. H. Neims (University of Florida, Gainesville, FL). All materials and reference standards for the autoradiographic analysis of testosterone and testosterone metabolites were obtained from previously cited sources (19).

7-Ethoxycoumarin, 7-hydroxycoumarin, and dimethylnitrosamine were obtained from Aldrich. 2-Methyl-1,2-ol-3-pyridyl-1-propanone, 5,5-dimethyl-1,3-cyclohexanedione, and all other biochemicals were obtained from Sigma.

Animals. Male and female C3H/HeJ mice (8 weeks old, 20–25 g) were purchased from Jackson Laboratories, Bar Harbor, ME. Mice of the same sex were housed in groups of up to six animals in wire mesh cages. The animals were maintained on a 12-hr light/12-hr dark photoperiod and were allowed both food (Purina Rodent Chow) and water *ad libitum*. All mice were 11–14 weeks of age at the time of study. The administration of testosterone propionate to female mice was according to the protocol described by Swank *et al.* (20) for the induction of female mouse renal glucuronidase activity. Female mice were given subcutaneous injections of testosterone propionate (10 mg/0.2 ml of olive oil) on days 0 and 1 and then were subsequently killed on day 7. Controls received oil only.

Preparation of microsomes. Renal microsomes were prepared by pooling six kidneys obtained from three mice into a single sample. Mice were killed by decapitation between 8:00 and 10:00 a.m., and kidneys were quickly excised, and kidney capsule and fatty tissue were carefully removed. The combined kidneys were weighed and then minced and homogenized in 5 volumes of ice-cold 250 mM potassium phosphate, pH 7.40, 150 mM potassium chloride in a motor-driven Teflon-glass Potter-Elvehjem homogenizer (approximately 10 passes). Washed renal microsomes were prepared by differential centrifugation (21). The protein content of microsomal suspensions was determined by the procedure of Lowry *et al.* (22). Cytochrome P-450 concentrations were determined by the method of Ohno *et al.* (21) for the measurement of renal microsomal cytochrome P-450 using the CO-induced difference spectrum of succinate-treated renal microsomes as baseline and a molar extinction coefficient of 91 mM^{-1} cm^{-1}. The possibility of contaminating hemoglobin and mitochondria in renal microsomal preparations necessitates the use of washed, succinate-reduced, renal microsomes for cytochrome P-450 determination.

Oxidative metabolism of model substrates. The catalytic activity of renal microsomes was determined under conditions in which metabolism of all substrates was proportional to microsomal protein concentration and time of incubation.

Steroid incubation and extraction conditions were similar to those described previously (19), except for substrate concentrations which were 100 μM. The low levels of female steroid hydroxylase activity required a 3-fold increase in all incubation and extraction volumes or in the specific activity of the labeled substrate for the metabolism of progesterone or testosterone, respectively, when using female renal microsomes. 19-OH progesterone (0.75 μg) was added after methylene chloride addition to each progesterone incubation mixture as an internal standard.

Testosterone 15α- and 16α-hydroxylations were determined by separation of metabolites by the radiochromatographic procedure described by Ford *et al.* (23) for the isolation and gas chromatographic-mass spectrometric identification of testosterone metabolites formed by mouse hepatic microsomes. Because of the restricted spectrum of testosterone metabolites formed by renal microsomes, only TLC in one direction was required for adequate separation of hydroxylated products. The dried methylene chloride extract, which contained over 98% of radioactivity, was dissolved in 50 μl of ethyl acetate. Aliquots (25 μl) of this extract and appropriate standards were applied to scored methanol-washed 20 × 20-cm Silica Gel $60F_{254}$ precoated 0.2-mm plastic-backed thin layer chromatography plates. The plates were developed three times in ether/benzene/methanol, 55/44/0.5 (v/v) in unlined tanks.

After chromatography, the radiolabeled metabolites of testosterone were located by autoradiography on x-ray film. Metabolites were identified by comigration with unlabeled reference standards which were visualized under UV light. The radioactive spots corresponding to 15α- and 16α-hydroxytestosterone were cut out and the radioactivity quantitated by liquid scintillation counting.

Progesterone 15α- and 16α-hydroxylations were determined by separation of metabolites by high-pressure liquid chromatography. All high-pressure liquid chromatography analyses were performed with a Waters model 204 liquid chromatograph equipped with two Waters model 6000A pumps, a Waters model 720 system controller, a Waters model 710B automatic injector system, and a Waters model 730 data module. The dried methylene chloride extract, containing added internal standard, was dissolved in 200 μl of acetonitrile, and injections of 25 μl were made for high-pressure liquid chromatography analysis. Progesterone metabolites formed by renal microsomes were resolved using a Waters μBondapak C18 column, a mobile phase consisting of a water:acetonitrile gradient and a flow rate of 2.0 ml/min. The column was initially eluted with a convex gradient, No. 5, from 25–32% acetonitrile over 12 min, followed by another No. 5 gradient to 45% aceto-

nitrile by 18 min. After 20 min, the unmetabolized progesterone was eluted off with 65% acetonitrile. All chromatographic separations were performed at ambient room temperature (22–24°), and column effluents were monitored at 254 nm using a Waters model 440 dual beam UV-visible absorbance detector. Metabolites were quantitated by integrated peak areas using the Waters model 730 data module and standard curves of reference standards constructed under identical extraction conditions as the unknown samples. Metabolites were identified by comparison of retention times with reference standards in the chromatographic system described above and by comigration of radiolabeled metabolites of progesterone with unlabeled reference standards using the radiochromatographic procedure described above for testosterone.

Lauric acid ω and (ω-1)-hydroxylations were determined by modifications of the procedure described by Orton and Parker (24) using a 5-μm Radial-PAK C18 column (100 × 8 mm, inner diameter, Waters Associates, Inc.) and a Waters Z-module radial compression separation system. The column was eluted isocratically with acetonitrile/water/glacial acetic acid (62.5:36.5:1, v/v) for 25 min at a flow rate of 1.0 ml/min. Lauric acid was subsequently eluted off with 100% acetonitrile by 35 min. The radiolabeled 11- and 12-hydroxylauric acids were detected, quantitated, and identified as described by others (24).

Dimethylnitrosamine demethylation was determined by measuring the rate of formation of [^{14}C]formaldehyde (25). As reported previously (25), the limits of sensitivity of this radiometric method are set by the degree of contamination of [^{14}C]dimethylnitrosamine with [^{14}C]formaldehyde. Measurement of low levels of DMNd[1] activity in female renal microsomes required prior removal of [^{14}C]formaldehyde contamination by Dowex-1-bisulfite chromatography of the [^{14}C]dimethylnitrosamine as described (18). The reaction was terminated by the addition of 0.3 ml of 1.0 M sodium acetate buffer, pH 4.5, 0.02 ml of 0.1 M formaldehyde solution, and 1.0 ml of a 10 mM aqueous solution of dimedon, and then placed on ice. Formation of the dimedon derivative and extraction of formaldemethone into hexane was as described previously (25) except instead of an additional extraction of the hexane layer with water, a 3.0-ml aliquot of the hexane phase was evaporated to dryness under N_2. Scintillation cocktail was then added to the dried hexane extracts for quantitation by scintillation spectroscopy.

Ethoxycoumarin *O*-deethylation was determined using the improved fluorometric assay described by Greenlee and Poland (26).

RESULTS

Consistent with previous findings (16), a 4-fold sex difference was observed in the cytochrome P-450 concentration of mouse renal microsomes. The cytochrome P-450 concentration in male renal microsomes (403 ± 39 pmol·mg^{-1}), with a Soret peak maximum for the reduced cytochrome P-450·CO complex of about 450 nm, was 40–50% of that normally found in hepatic microsomes. In contrast, female renal microsomes were characterized by lower concentrations of cytochrome P-450 (93 ± 7 pmol·mg^{-1}), only 10–15% of that found in hepatic microsomes, and a corresponding Soret peak maximum for the reduced CO complex of 452 nm.

Metabolism of endogenous substrates. Large sex differences in testosterone 15α- and 16α-hydroxylase activities have been reported using renal microsomes from AKR/J mice (16). To further characterize the sex specificity of mouse renal microsomes with regard to some endogenous substrates, hydroxylase activities toward testosterone, progesterone, and lauric acid were compared using renal microsomes prepared from male and female C3H/HeJ mice.

[1] The abbreviations used are: DMNd, dimethylnitrosamine demethylase; ECOD, 7-ethoxycoumarin *O*-deethylase.

Table 1 summarizes the various regiospecific hydroxylase activities for male and female renal microsomes. For male-female comparison, hydroxylase activities are also expressed in terms of total cytochrome P-450 (i.e., apparent turnover values) to compensate for the large sex differences in renal microsomal cytochrome P-450 concentration. As seen in Table 1, greater rates for 15α-hydroxylation were obtained when progesterone, rather than testosterone, served as the substrate with either male or female renal microsomes. The sex difference in renal microsomal 15α-hydroxylase activity was approximately 8-fold with either testosterone or progesterone. This difference was much greater than the sex difference in P-450 concentration as reflected by turnover values which were 2-fold higher for male renal microsomes. In contrast, differences in the rates of 16α-hydroxylation between the two steroid substrates were sex specific. The rate of 16α-hydroxylation was three times greater for progesterone than for testosterone when male renal microsomes were used. Conversely, the rate for the 16α-hydroxylation of progesterone was only one-half that of testosterone when female renal microsomes were used. Greater male specificity for the 16α-hydroxylation of progesterone is indicated by an increase in the observed sex differences for 16α-hydroxylase activity from approximately 3-fold for testosterone to 18-fold for progesterone and by a 4-fold male predominance in apparent turnover values for the 16α-hydroxylation of progesterone. In contrast, female specificity for the 16α-hydroxylation of testosterone is reflected by a female predominance in the apparent turnover values for this activity.

Similar to the differences observed in steroid 15α-hydroxylase activity, male laurate 11-hydroxylase activity was 8-fold higher than the corresponding female activity resulting in a 2-fold greater turnover value for male renal microsomes. Surprisingly, no sex difference was observed in the high rate (nanomole range *versus* picomole for the other substates) of lauric acid 12-hydroxylation by mouse renal microsomes. The combination of high laurate 12-hydroxylase activity and low cytochrome P-450 concentration resulted in 4- to 5-fold greater turnover values for female renal microsomes. The 12:1 product ratio (12-OH to 11-OH) for lauric acid metabolism by female renal microsomes is strikingly different from the 1:1 ratio obtained with male renal microsomes.

Xenobiotic metabolism. 7-Ethoxycoumarin-O-deethylation was assayed at two substrate concentrations because analyses of kinetic plots of 7-ethoxycoumarin-O-deethylase activity in mouse hepatic microsomes have revealed high and low affinity components (26). As seen in Table 2, when assayed at 500 μM, male renal ECOD activity was 3-fold greater than female ECOD activity, although this difference was less than that observed in cytochrome P-450 concentration as indicated by an approximately 2-fold greater turnover value for female renal microsomes. These results were similar to those obtained only for the 16α-hydroxylation of testosterone. Lowering the substrate concentration to 5 μM resulted in a decreased sex difference for mouse renal ECOD activity. The diminished sex difference was largely the

TABLE 1

Sex differences in the rates for the regiospecific hydroxylations of three endogenous substrates using renal microsomes prepared from C3H/HeJ mice

Values represent the means ± S.D. of duplicate assays on renal microsomes from three to four groups of mice (i.e., pooled kidneys from three mice per group). Numbers in parentheses represent the male/female ratio. Asterisks indicate statistical differences between sexes ($p < 0.05$; Student's *t* test).

	Hydroxylase activity					
	Testosterone		Progesterone		Lauric acid	
	15α-OH	16α-OH	15α-OH	16α-OH	11-OH	12-OH
	pmol/min/mg		*pmol/min/mg*		*nmol/min/mg*	
Male	260 ± 26	37 ± 7	550 ± 28	106 ± 13	1.48 ± 0.07	1.75 ± 0.31
Female	34 ± 2	13 ± 1	58 ± 6	6 ± 1.5	0.18 ± 0.02	2.09 ± 0.49
	(7.6)*	(2.8)*	(9.5)*	(17.7)*	(8.2)*	(0.84)
	Apparent turnover value					
	15α-OH	16α-OH	15α-OH	16α-OH	11-OH	12-OH
	pmol/min/nmol P-450		*pmol/min/nmol P-450*		*nmol/min/nmol P-450*	
Male	645 ± 11	91 ± 16	1370 ± 62	266 ± 44	3.61 ± 0.39	4.30 ± 0.99
Female	368 ± 8	135 ± 10	629 ± 19	68 ± 12	1.65 ± 0.18	19.31 ± 3.69
	(1.8)*	(0.67)*	(2.2)*	(3.9)*	(2.2)*	(0.22)*

TABLE 2

Sex differences in the O-deethylation of 7-ethoxycoumarin and N-demethylation of dimethylnitrosamine using mouse renal microsomes

Values represent the means ± S.D. of duplicate assays on renal microsomes from three groups of mice (i.e., pooled kidneys from three mice per group). Numbers in parentheses represent the male/female ratio. Asterisks indicate statistical differences between sexes ($p < 0.05$; Student's *t* test).

	7-Ethoxycoumarin *O*-deethylase				Dimethylnitrosamine *N*-demethylase			
	500 μM	5 μM	500 μM	5 μM	2 mM	20 μM	2 mM	20 μM
	pmol/min/mg microsomal protein		*pmol/min/nmol P-450*		*pmol/min/mg microsomal protein*		*pmol/min/nmol P-450*	
Male	97 ± 6	16 ± 2	223 ± 29	36 ± 6	180 ± 13	8 ± 0.7	447 ± 20	20 ± 0.6
Female	32 ± 3	12 ± 0.5	402 ± 30	146 ± 4	22 ± 5	1 ± 0.1	239 ± 56	10 ± 0.6
	(3.0)*	(1.3)*	(0.55)*	(0.25)*	(8.2)*	(8.0)*	(1.9)*	(2.0)*

result of the greater decrease in male ECOD activity which was only 17% of the 500 μM activity compared to female ECOD activity which was still 38% of the 500 μM activity when assayed at an ethoxycoumarin concentration of 5 μM. At the low substrate concentration, female turnover values for renal ECOD activity were 4-fold greater than for male renal microsomes, indicating the presence of a high affinity enzyme in female mouse renal microsomes. Because other substrate concentrations were not examined, it is not known whether mouse renal ECOD activity represents the cumulative effects of more than a single cytochrome P-450 isozyme as has been reported for hepatic microsomes.

The demethylation of dimethylnitrosamine (DMNd) is another enzyme activity for which multiple K_m values have been reported using hepatic microsomes (27). In contrast to the results obtained using ethoxycoumarin as substrate, the sex difference in mouse renal DMNd activity remained constant at high and low concentrations of dimethylnitrosamine (Table 2). Male DMNd activity was approximately 8-fold higher than female renal DMNd activity when assayed at a 2 mM substrate concentration. We have recently examined the kinetics of renal microsomal DMNd activity in male and female mice[2] and, consistent with the above data, have found similar K_m values for male and female microsomes (0.5 and 0.4 mM, respectively) and a 10-fold difference in the V_{max}.

Androgen induction. In recent studies with mouse renal microsomes utilizing testosterone as a substrate, low levels of female renal 15α- and 16α-hydroxylase activities were not induced by testosterone propionate pretreatment although a significant increase was observed in cytochrome P-450 concentration (16). This refractoriness to androgen induction may represent a general feature of the female renal monooxygenase system or may be a unique property of the steroid monooxygenase system only. To explore these possibilities, the effects of testosterone propionate administration on the hydroxylations of progesterone as well as on the demethylation of dimethylnitrosamine and the hydroxylations of lauric acid were determined.

The data in Fig. 1 for C3H/HeJ female renal microsomes are expressed as a percentage of the corresponding male values which are presented in the figure legend. Female cytochrome P-450 content was induced 2.5-fold to 64% of the male level. Accompanied by the increase in cytochrome P-450 concentration was a shift in the Soret peak maximum from 452 to 450 nm. Similar to previous results obtained with testosterone, progesterone 15α- and 16α-hydroxylase activities were not signifi-

[2] R. L. Hawke and R. M. Welch, manuscript in preparation.

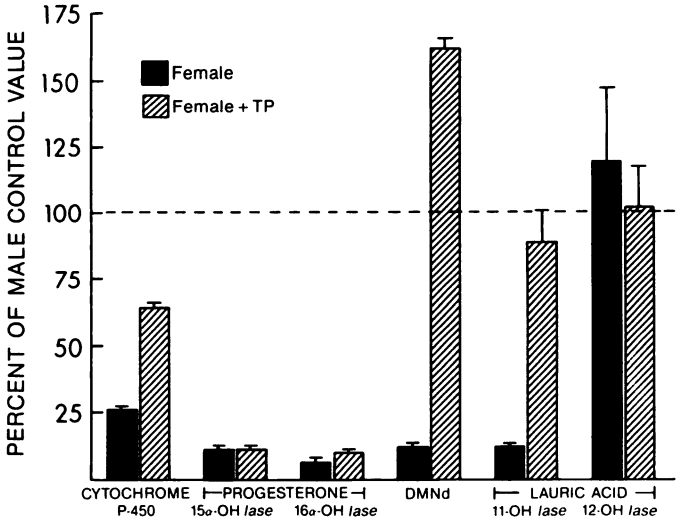

FIG. 1. *The effect of testosterone propionate pretreatment on cytochrome P-450 and on several monooxygenase activities of female renal microsomes*

Female mice were given subcutaneous injections of testosterone propionate (*TP*) (10 mg/0.2 ml of olive oil) on days 0 and 1, and then were subsequently killed on day 7. Controls received oil only. *Dashed line* represents respective male values (100%). *Solid bars* represent sesame oil-treated female values and *hatched bars* represent testosterone propionate-treated female values. Values represent the means ± S.D. for four female renal microsomal preparations (three female mice per preparation). The specific activities and standard deviations for male control values were as follows: 0.42 ± 0.03 nmol of cytochrome P-450/mg of microsomal protein; 415 ± 26 and 83 ± 5 pmol of hydroxyprogesterone/min/mg for progesterone 15α- and 16α-hydroxylase activities, respectively; 140 ± 11 pmol of HCHO/min/mg for dimethylnitrosamine demethylation (DMNd); and 1.48 ± 0.07 and 1.75 ± 0.31 nmol of hydroxylaurate/min/mg for 11- and 12-hydroxylations of lauric acid, respectively.

cantly induced by androgen pretreatment. In analogous experiments with dihydrotestosterone and AKR/J and BALB/cJ female mice, 15α- and 16α-hydroxylase activities were never induced greater than 10% of their respective control male values (data not shown). In contrast, female renal DMNd activity was induced 14-fold to a value that was 60% greater than the corresponding male control activity. Similarly, the low female rate of lauric acid 11-hydroxylation was induced 8-fold to near male levels, while the high rate of 12-hydroxylation remained unchanged by androgen pretreatment.

Inhibition studies. In addition to the results in Fig. 1 which indicate heterogeneity of the mouse renal monooxygenase system, inhibition studies of male renal progesterone hydroxylase activities also revealed exquisite inhibitor and substrate specificities. The addition of either 20 mM dimethylnitrosamine or 100 μM lauric acid did not produce any significant inhibitions of either progesterone 15α- or 16α-hydroxylase activity of male renal microsomes (Table 3). In contrast, addition of 100 μM testosterone to the progesterone assay resulted in differential inhibition of renal 15α- or 16α-hydroxylase activities by 68 and 44%, respectively. Metyrapone, a classical inhibitor of many cytochrome P-450-dependent monooxygenase activities also differentiated the two progesterone hydroxylase activities. As shown in Table 3,

TABLE 3

The effects of dimethylnitrosamine (DMN), lauric acid, testosterone, and metyrapone on the 15α- and 16α-progesterone hydroxylase assay using male mouse renal microsomes

Data are expressed as percentages of the control values. Values represent the means ± S.D. of duplicate assays on renal microsomes from three groups of mice (pooled kidneys from three mice per group). Asterisks indicate statistical differences from control ($p < 0.05$; Student's t test).

	Progesterone hydroxylase activity	
	15α (597 ± 67[a])	16α (90 ± 10[a])
	% of control hydroxylase activity	
Control assay	100.0	100.0
+ 20 mM DMN	95.4 ± 5.5	96.9 ± 9.4
+ 100 μM lauric acid	94.1 ± 5.2	91.6 ± 5.6
+ 100 μM testosterone	31.7 ± 3.5*	55.6 ± 6.0*
+ Metyrapone		
5×10^{-6} M	42.9 ± 1.8*	92.2 ± 8.6
5×10^{-5} M	8.3 ± 0.2*	78.6 ± 11.8

[a] pmol/min/mg/microsomal protein.

metyrapone produced a concentration-dependent inhibition of renal 15α-hydroxylase activity only. At 5×10^{-6} and 5×10^{-5} M metyrapone, 15α-hydroxylase activity was reduced by 57 and 92%, respectively, although no significant effect was observed on 16α-hydroxylase activity at either metyrapone concentration.

DISCUSSION

The isolation of a number of inducible cytochrome P-450 isozymes from liver microsomes of rat and rabbit (for reviews see Refs. 1 and 3) as distinct proteins with different chemical and physical properties has established the multiplicity of mammalian hepatic microsomal cytochrome P-450. In contrast, only limited information is availble on the multiplicity and regulation of cytochrome P-450 residing in the microsomal fraction of extrahepatic tissues. This is particularly evident in the kidney where recent attention has focused almost exclusively on the localization of inducible cytochrome P-450-dependent enzyme systems in the rabbit kidney cortex (15, 28), although definitive proof of renal P-450 multiplicity was only recently provided by the separation of inducible forms of cytochrome P-450 from rabbit renal cortex microsomes (29).

The 4- to 5-fold sex difference in mouse renal cytochrome P-450 concentration likely results from discrete quantitative differences in sex-specific P-450 isozymes in mouse renal microsomes as has been shown in rat hepatic microsomes (5, 30). This is suggested by the difference in the Soret peak maximums of the reduced cytochrome P-450·CO complexes, 450 *versus* 452 nm, for male and female renal microsomes, respectively. The administration of androgen to female mice produced a shift in the Soret peak maximum for female renal microsomes. This shift in the Soret peak from 452 nm to 450 nm, a wavelength which is characteristic of male renal microsomes, was accompanied by a partial elevation in cytochrome P-450 concentration to male levels. Orrenius

et al. (31) have shown that the presence of reduced cytochrome oxidase in renal microsomes produces a shift in the absorption peak for the reduced P-450·CO complex to higher wavelengths. They were able to compensate for this effect by incubating renal microsomes with succinate in the presence of carbon monoxide and reported an absorption peak at 452 nm for the reduced P-450·CO complex of rat renal microsomes. We have applied a similar method described by Ohno *et al.* (21) for the determination of microsomal cytochrome P-450 in renal tissue and concur with their report of an absorption peak at 450 nm for male mouse renal microsomes. Although these investigators did not examine female mouse renal microsomes, they did report a peak absorption maximum of 452 nm for the reduced P-450·CO complex of rat renal microsomes.

Recently it has been shown that male mouse renal microsomes yield rates for the 15α-hydroxylation of testosterone which are comparable to that of hepatic microsomes, while rates for 16α-hydroxylation are approximately 10-fold lower, and other products of hepatic testosterone metabolism are not observed with male renal microsomes (16). In contrast, female mouse renal microsomes are more similar to rat kidney cortex microsomes which are characterized by a lower concentration of cytochrome P-450, a Soret peak maximum of 452 nm, and no measurable testosterone hydroxylase activity (12). It is not known whether this renal male-specific steroid 15α-hydroxylase is identical to the 15α-hydroxylase recently purified from mouse liver microsomes (32). In hepatic microsomes, the sex differences observed in 15α-hydroxylase activity are strain dependent with female microsomes demonstrating 4-to 5-fold greater rates of activity (33).

The existence of sex-specific forms of cytochrome P-450 in mouse renal microsomes is supported by the sex differences observed in the regio- and stereo-specific hydroxylations observed in this study. These differences suggest the presence of major male-specific P-450 isozymes in mouse kidney resulting in higher P-450 concentrations, a Soret peak maximum of 450 nm, and, for example, in high rates of steroid 15α-hydroxylase activity. Androgen induction studies provided the most insight into mouse renal heterogeneity. The renal microsomal steroid hydroxylating system was differentiated by the inability of androgen pretreatment to induce levels in female mice although high levels of 15α-hydroxylase activity are found in male renal microsomes. These results suggest for the first time the possibility of extrahepatic neonatal imprinting of steroid hydroxylase activity similar to that observed for many hepatic enzyme activities in the rat (34, 35). In contrast, DMNd and laurate 11-hydroxylase activities were androgen inducible while laurate 12-hydroxylase activity was little affected by androgen pretreatment. These results also provide evidence for the involvement of at least two forms of renal cytochrome associated with the 11- and 12-hydroxylations of lauric acid by mouse kidney microsomes. It is generally accepted that while two forms participate in lauric acid hydroxylation by rat hepatic microsomes, only a single form catalyzes these reactions in rat kidney cortex microsomes (36).

Support for separate progesterone 15α- or 16α-hydroxylases derives from the metyrapone inhibition studies and the differential inhibition effect of testosterone on these two enzyme activities. The results of our inhibition studies also suggest minimal overlap in specificity toward the substrates examined here and further distinguish the steroid monooxygenase system. Only testosterone effectively inhibited progesterone 15α- or 16α-hydroxylase activities of male renal microsomes. DMNd activity and lauric acid 11-hydroxylase activity were the only androgen-inducible monooxygenase activities in female renal microsomes. Studies attempting to elucidate the relationship between these two classically divergent monooxygenase activities are currently in progress. Definitive proof of mouse renal cytochrome P-450 heterogeneity rests on the purification of these forms and the subsequent reconstitution of their associated catalytic activities.

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