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EXPRESSION OF HEPATIC MICROSOMAL CYTOCHROME P450s AS ALTERED BY UREMIA

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Abstract—The proportions of different hepatic microsomal cytochrome P450s expressed in uremic rats were studied with specific antibodies and with a steroid hydroxylase assay. In male uremic rats, the hepatic levels of P450 2C11, a male-specific form, and 3A2, a male-dominant form, were decreased to about 30% at 5 weeks after the induction of uremia. These changes were paralleled by decreases in the activities of testosterone 2α-, 16α-, and 6β-hydroxylation. The level of P450 2A1, abundant in immature rats, was increased 2-fold by uremia and accompanied by an increase in testosterone 7α-hydroxylation activity. The levels of P450 2C6 and 2E1 were not changed by uremia. The levels of male-specific and male-dominant forms such as P450 2C11 and 3A2 are affected by the serum level of testosterone, which was decreased in the male rats with uremia. Therefore, castrated rats were prepared to compare the effects of testosterone on hepatic cytochrome P450s in uremic rats with those in castrated rats. When testosterone was administered to the castrated rats, the decreased levels of both P450 2C11 and 3A2 returned to normal. However, the administration of testosterone to the uremic rats did not prevent the decrease in the levels of these P450s. In female rats, changes in the levels of cytochrome P450s were not as great during uremia as those in male uremic rats. The level of P450 2C12, a female-specific form, was not changed; the level of P450 2A1 was increased by 50%, that of 3A2 which is barely detected in female rats was increased by 60%, and that of 2E1 was increased by 25%. These results suggested that the changes in the hepatic levels of cytochrome P450s were affected by factors other than testosterone in uremic rats.

Uremia can cause a variety of functional abnormalities in almost every organ of the body. The metabolism of vitamins, hormones, proteins, lipids, steroids, and drugs is altered in the uremic state. For example, abnormalities in the biological half-life of several drugs metabolized by liver microsomal enzymes occur in uremic subjects [1, 2]. Despite adjustment of the dosage to compensate for the reduced renal clearance, the frequency and severity of adverse drug reactions are greater in patients with renal failure than in patients without renal failure [3, 4].

The hepatic monooxygenase system including cytochrome P450 metabolizes many exogenous and endogenous substances, including steroids and fatty acids [5–7]. The metabolism of chemicals by the hepatic monooxygenase system is altered by diseases such as diabetes and hypertension, because of changes in the levels of individual cytochrome P450s [8–11]. Changes in hormonal levels caused by diseases affect the levels of cytochrome P450. The proportions of different cytochrome P450s in hepatic microsomes are regulated by several factors, including age, sex, altered physiological states, and administration of drugs and chemicals [8–13]. Some forms of cytochrome P450 are expressed sex-specifically in the rat liver, so that there is a sex-related difference in the properties of hepatic microsomal cytochrome P450s. Male-specific forms are affected by the serum level of testosterone. Uremic rats can develop hypogonadism [14]. It is therefore of interest to find out how cytochrome P450 in the liver is changed and regulated in conditions such as uremia. Leber and Schütterle [15] found that the activities of hepatic microsomes towards some chemicals and the amount of cytochrome P450 measured photometrically are decreased by uremia. Patterson and Cohn [16] reported a similar decrease in cytochrome P450-dependent activities, but found that the activities of 6-methylmercaptopurine riboside *S*-demethylase and β-naphthylacetate esterase and the amounts of cytochrome b_5 and NADPH–cytochrome P450 reductase are unaltered. The role of individual cytochrome P450s that metabolize drugs in uremic rats has not been studied, although at least twenty forms have been isolated from the rat liver and characterized biochemically [12, 17, 18].

In this study, we investigated the metabolic activities of hepatic microsomes and the changes in the hepatic levels of different cytochrome P450s in rats, using a surgically produced model of uremia.

MATERIALS AND METHODS

Animal treatment. Eight-week-old inbred male and female Sprague–Dawley rats (Clea Japan, Inc., Tokyo) were used. Uremia was brought about in the rats by the method of Ormrod and Miller [19] by resection of both the upper and lower poles of the left kidney and then removal of one-third of the

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remaining cortical tissue from the exterior lateral aspect of this kidney. The right kidney was removed 7 days later. As a control, sham-operated rats were prepared by exposure of the left kidney, decapsulation, and removal of thin slices of the cortical tissue from the surface of the kidney. Seven days later, the right kidney was surgically exposed. In a separate experiment, 8-week-old Sprague–Dawley rats were castrated. Testosterone was given to uremic or castrated rats by subcutaneous injection of testosterone propionate (10 mg/kg in corn oil) three times a week from the third to fifth week (for a total of nine times) after the induction of uremia or castration. Serum urea nitrogen and serum creatinine levels were assayed with a Gemstar autoanalyzer (Tateishi Inc., Tokyo, Japan). The serum testosterone level was assayed by a specific radioimmunoassay with a commercial kit (Testosterone ^{125}I kit, Sorin Biomedica, S.p.A., Italy).

Preparation of microsomes. The livers were homogenized with a Potter Teflon homogenizer in 3 vol. of 1.15% KCl, 1 mM EDTA, and 0.25 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, MO). Microsomal fractions were obtained by differential centrifugations. The microsomal pellets obtained were suspended in 0.1 M potassium phosphate buffer (pH 7.4), 1 mM EDTA, 1 mM dithiothreitol, and 30% glycerol to a concentration of 30–50 mg protein/mL and stored at −90°.

Purification of cytochrome P450s and preparation of antibodies. P450 2C11 (UT-2) was purified from untreated male rats, and P450 3A2 (PB-1) and 2C6 (PB-2) were purified from rats treated with phenobarbital as reported previously [17]. P450 2A1 (IF-3) was purified from immature female rats [20]. P450 2E1 (DM) was purified from rats with diabetes caused by streptozotocin [9]. P450 2C12 (F-2) was purified from untreated adult female rats [21]. The nomenclature of cytochrome P450s used in this study is that of Nebert *et al.* [22]. Antibodies against the purified cytochrome P450s (P450 2C11, 3A2, 2C6, 2A1, 2C12, and 2E1) were raised as described previously in a female Japanese White rabbit obtained from Biotech (Saga, Japan) [21]. Characterization of the antibodies has been described elsewhere [23].

Immunochemical study. The immunoblotting and immunochemical assays of individual cytochrome P450 isozymes were done as reported previously [23]. After sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis with a 7.5% polyacrylamide gel by the method of Laemmli [24], the proteins were transferred from the gel to a nitrocellulose sheet (Bio-Rad Laboratories, Richmond, CA) in a buffer containing 100 mM Tris, 192 mM glycine, and 20% methanol [25]. The nitrocellulose membrane was treated with the antibody and stained with a Vectastain ABC kit (Vector Laboratories, Burlingame, CA). Immunochemical assays were done by densitometry of the stained nitrocellulose sheets.

Other methods. Aminopyrine N-demethylation activity, 7-ethoxycoumarin O-dealkylation activity, and testosterone hydroxylation activity were measured as described previously [21]. Protein concentrations, cytochrome P450 content, cytochrome b_5 content, and NADPH–cytochrome P450 reductase activity were measured by methods reported previously [26–29].

RESULTS

Blood biochemistry and hepatic content of cytochrome P450 in uremic rats and castrated rats. Test results for blood biochemistry and the hepatic content of cytochrome P450 are shown in Table 1. Serum urea nitrogen and serum creatinine reached a peak 1 week after uremia was brought about. At 5 weeks, serum urea nitrogen and serum creatinine were 101.7 ± 8.8 and 1.52 ± 0.13 mg/dL, respectively, in the male uremic rats and 86.8 ± 19.6 and 1.56 ± 0.42 mg/dL, respectively, in the female uremic rats. The specific content of cytochrome P450 was decreased in the male uremic rats. Although this level gradually increased with time (1, 3, and 5 weeks after the induction of uremia), it was still significantly low at 5 weeks. The specific contents of cytochrome b_5 and NADPH–cytochrome P450 reductase were unchanged in these rats. There was no change in the specific content of cytochrome P450 in the female uremic rats.

The serum testosterone level was low in the male uremic rats. Some forms of cytochrome P450 that are expressed sex-specifically in the liver are affected by the serum level of testosterone [30]. To identify the effects of testosterone on the regulation of the expression of cytochrome P450, castrated rats were prepared as a model of those with decreased serum testosterone level. The specific content of cytochrome P450 was decreased in the castrated rats to the same degree as in the male uremic rats. By the administration of testosterone to the castrated rats, the specific content of cytochrome P450 was restored to the level in the control rats, but the administration of testosterone to the male uremic rats did not affect the decrease.

Metabolic activity of hepatic microsomes in uremic rats and castrated rats. The metabolic activities of hepatic microsomes are shown in Table 2. In the male uremic rats, the aminopyrine N-demethylation activity and the 7-ethoxycoumarin O-dealkylation activity of hepatic microsomes were diminished by uremia, although these activities increased with time. Testosterone hydroxylation is one index of the changes in the different forms of cytochrome P450 in hepatic microsomes [31]. In our study, testosterone 2α- and 16α-hydroxylation activities were decreased in the male uremic rats, suggesting that the level of P450 2C11 in hepatic microsomes may be decreased [31]. Testosterone 2β- and 6β-hydroxylation activities were also decreased in the male uremic rats, suggesting that the level of P450 3A2 may be decreased [32]. Testosterone 7α-hydroxylation activity was increased in the male uremic rats, suggesting that the level of P450 2A1 may be increased [20]. There were no changes in testosterone 6α-, 15α-, or 16β-hydroxylation activities (data not shown). In the castrated rats, the aminopyrine N-demethylation activity and the 7-ethoxycoumarin O-dealkylation activity of hepatic microsomes were decreased. Testosterone 2α-, 16α-, and 6β-hydroxylation activities were also decreased in these rats to the same levels as in the male uremic rats. These

Table 1. Alterations in blood biochemistry and the contents of total cytochrome P450, cytochrome b_5 and NADPH–cytochrome P450 reductase of uremic and castrated rats

	Serum urea nitrogen (mg/dL)	Serum creatinine (mg/dL)	Serum testosterone (ng/mL)	P450 (nmol/mg)	b_5 (nmol/mg)	Fp_2 (unit/mg)
Male control	20.4 ± 1.6	0.58 ± 0.07	2.52 ± 1.21	0.59 ± 0.09	0.44 ± 0.06	0.14 ± 0.01
Uremia (1 week)	132.8 ± 11.5*	1.98 ± 0.28*	0.25 ± 0.09*	0.35 ± 0.07*	0.42 ± 0.06	0.12 ± 0.01
Uremia (3 weeks)	115.7 ± 12.7*	1.81 ± 0.22*	0.48 ± 0.11*	0.40 ± 0.05*	0.47 ± 0.06	0.12 ± 0.01
Uremia (5 weeks)	101.7 ± 8.8*	1.52 ± 0.13*	0.55 ± 0.23*	0.42 ± 0.03*	0.47 ± 0.07	0.12 ± 0.01
Uremia treated with testosterone	115.3 ± 13.4*	1.62 ± 0.12*	7.64 ± 1.53*†	0.49 ± 0.03‡	0.51 ± 0.09	0.14 ± 0.01
Male castration	22.1 ± 1.7	0.66 ± 0.07	<0.1*	0.41 ± 0.02*	0.44 ± 0.05	0.12 ± 0.01
Castration treated with testosterone	22.1 ± 1.6	0.61 ± 0.11	11.32 ± 0.47*	0.62 ± 0.06	0.47 ± 0.06	0.14 ± 0.01
Female control	22.2 ± 2.7	0.54 ± 0.13	<0.1	0.56 ± 0.07	0.61 ± 0.11	0.14 ± 0.01
Uremia (5 weeks)	86.8 ± 19.6*	1.56 ± 0.42*	<0.1	0.54 ± 0.05	0.65 ± 0.08	0.14 ± 0.01

Assay was done on five or six different preparations of microsomes and the values are expressed as means ± SD. Key: b_5, cytochrome b_5; and Fp2, NADPH–cytochrome P450 reductase.

* Significantly different from control, $P < 0.01$.

† Significantly different from male uremic rats (5 weeks), $P < 0.01$.

‡ Significantly different from control, $P < 0.05$.

Table 2. Alterations in the catalytic activity of rat hepatic microsomes of uremic and castrated rats

	Aminopyrine	7-Ethoxycoumarin	Testosterone 2α	Testosterone 2β	Testosterone 6β	Testosterone 7α	Testosterone 16α
Male control	7.21 ± 0.95	2.74 ± 0.44	1.13 ± 0.23	0.35 ± 0.12	1.48 ± 0.39	0.09 ± 0.01	1.73 ± 0.35
Uremia (1 week)	4.56 ± 0.97*	1.24 ± 0.28*	0.25 ± 0.05*	0.11 ± 0.05*	0.32 ± 0.15*	0.09 ± 0.01	0.20 ± 0.12*
Uremia (3 weeks)	4.84 ± 0.93*	1.51 ± 0.21*	0.41 ± 0.21*	0.19 ± 0.07†	0.38 ± 0.15*	0.13 ± 0.01*	0.29 ± 0.18*
Uremia (5 weeks)	5.85 ± 0.91†	1.81 ± 0.25*	0.45 ± 0.22*	0.21 ± 0.07	0.39 ± 0.16*	0.13 ± 0.01*	0.56 ± 0.38*
Uremia treated with testosterone	6.01 ± 0.82	1.88 ± 0.28*	0.48 ± 0.08*	0.36 ± 0.08‡	0.61 ± 0.38*	0.13 ± 0.01*	0.52 ± 0.21*
Male castration	5.52 ± 0.88†	1.52 ± 0.24*	0.36 ± 0.12*	0.18 ± 0.11†	0.43 ± 0.19*	0.12 ± 0.01*	0.74 ± 0.15*
Castration treated with testosterone	7.51 ± 0.92	2.95 ± 0.45	1.42 ± 0.28	0.49 ± 0.15	1.96 ± 0.36	0.11 ± 0.01	1.61 ± 0.33
Female control	3.75 ± 0.85	1.08 ± 0.06	0.02 ± 0.01	0.04 ± 0.01	0.14 ± 0.01	0.23 ± 0.02	0.04 ± 0.01
Uremia (5 weeks)	3.80 ± 0.77	1.19 ± 0.12	0.02 ± 0.01	0.05 ± 0.01	0.16 ± 0.02	0.25 ± 0.02	0.04 ± 0.01

Assay was done on five or six different preparations of hepatic microsomes with duplicates, and the values are expressed as means ± SD in nanomoles of product per minute per milligram of microsomal protein. 2α, 2β, 6β, 7α, and 16α indicate the hydroxylation site of testosterone.

* Significantly different from control, $P < 0.01$.

† Significantly different from control, $P < 0.05$.

‡ Significantly different from male uremic rats (5 weeks), $P < 0.05$.

levels returned to normal with the administration of testosterone to the castrated rats. In the male uremic rats, testosterone 2β-hydroxylation activity was restored and 6β-hydroxylation activity seemed to be increased by testosterone administration, but the activities of aminopyrine N-demethylation, 7-ethoxycoumarin O-dealkylation, and testosterone 2α-, 7α-, and 16α-hydroxylation were not affected. Anti-P450 2A1, 2C11, or 3A2 antibodies almost completely inhibited 7α-hydroxylation, 2α- and 16α-hydroxylation, or 2β- and 6β-hydroxylation activities, respectively, of hepatic microsomes of control and uremic male rats (data not shown). Each hydroxylation activity of hepatic microsomes toward testosterone was dependent on the activity of individual cytochrome P450s. These metabolic activities were not altered by uremia in the female rats.

Hepatic level of forms of cytochrome P450 in uremic rats and castrated rats. The increase or decrease in certain microsomal activities depending on cytochrome P450 suggested that the level of one or more forms of cytochrome P450 may be decreased while that of other forms may be increased. Therefore, specific polyclonal antibodies were prepared against six cytochrome P450s to monitor the changes in these enzymes in hepatic microsomes caused by uremia. Table 3 shows the results of quantitative Western blots of the microsomal samples. Consistent with the decrease in testosterone 2α- and 16α-hydroxylation activities, the level of P450 2C11, a major male-specific form, was decreased in the male uremic rats as compared with that in the control rats. This change can explain the decrease in the aminopyrine N-demethylation activity of hepatic microsomes in the male uremic rats, as P450 2C11 has high aminopyrine N-demethylation activity [33]. P450 3A2 is a major form of cytochrome P450 in untreated male rats. The level of P450 3A2, constitutive testosterone 6β-hydroxylase [32], was decreased by uremia, consistent with the decrease in testosterone 6β-hydroxylation activity. P450 2C6 is present in both sexes [21], and its level was little changed by uremia. The level of P450 2E1, a form that is induced by diabetes or ethanol administration [10], was also little changed by uremia. P450 2A1 is present in the hepatic microsomes of both sexes and abundant in immature rats [20]. P450 2A1 has mostly testosterone 7α-hydroxlation activity in a reconstituted system and is important in testosterone 7α-hydroxylation in rat hepatic microsomes [20]. Consistent with the increase in microsomal 7α-hydroxylation activity, the level of P450 2A1 was increased by uremia. In the female uremic rats, almost no change was observed in the levels of cytochrome P450 2C12, a major female-specific form, and P450 2C6 as compared with those in the control rats. The level of P450 2A1 was increased by uremia, as in the male rats and the level of P450 3A2, which is barely detectable in female rats, was increased slightly in the female uremic rats, although increases of testosterone 6β- and 7α-hydroxylation activities (catalyzed by P450 3A2 and 2A1, respectively) were not observed. The discrepancy of hydroxylation activity and the level of cytochrome P450 was not explained. The level of P450 2E1 was also slightly increased. In the castrated male rats, cytochrome P450 2C11 and 3A2 levels were decreased, the P450 2A1 level was increased, and P450 2C6 and 2E1 levels were changed little (as in the male uremic rats), when compared with levels in the male control rats. After the administration of testosterone, however, the levels of P450 2C11 and 3A2 rose to levels higher than those in the control rats. These changes in the levels of each form of cytochrome P450 were consistent with those in testosterone hydroxylation activities. However, testosterone treatment only slightly affected the levels of P450 3A2 in the male uremic rats in contrast to results in the castrated rats.

DISCUSSION

Changes in hepatic microsomal cytochrome P450 levels in uremic rats have been monitored in catalytic studies of testosterone and immunoblotting studies done with specific antibodies. In our study, the levels

Table 3. Alterations in the levels of cytochrome P450 of rat hepatic microsomes of uremic and castrated rats

	3A2	2C6	2A1	2E1	2C11	2C12
Male control	113.5 ± 18.1	126.8 ± 16.4	11.9 ± 4.1	38.6 ± 11.5	355.2 ± 29.6	ND
Uremia (1 week)	20.8 ± 3.8*	115.1 ± 26.7	17.5 ± 4.6	27.1 ± 9.5	34.4 ± 15.1*	ND
Uremia (3 weeks)	26.5 ± 2.0*	146.3 ± 13.1	24.3 ± 6.0†	43.9 ± 10.5	44.6 ± 24.2*	ND
Uremia (5 weeks)	36.9 ± 6.0*	145.1 ± 38.2	23.6 ± 9.1†	47.6 ± 6.6	128.1 ± 32.1*	ND
Uremia treated with testosterone	62.6 ± 9.1*‡	153.3 ± 29.8	24.0 ± 7.5†	43.7 ± 3.5	165.3 ± 30.9*	ND
Male castration	39.7 ± 9.6*	148.9 ± 14.9	22.0 ± 6.8†	36.6 ± 2.5	219.3 ± 47.9*	ND
Castration treated with testosterone	161.1 ± 2.9*	147.2 ± 15.9	18.6 ± 5.5	38.2 ± 22.1	427.3 ± 31.5*	ND
Female control	10.4 ± 2.9	120.5 ± 15.2	31.1 ± 9.8	49.3 ± 5.4	ND	204.2 ± 17.2
Uremia (5 weeks)	16.4 ± 3.1†	132.4 ± 22.2	44.7 ± 6.1†	63.2 ± 8.7†	ND	232.2 ± 42.1

Levels of cytochrome P450s were assayed by densitometry of nitrocellulose immunoblotted from an SDS-polyacrylamide gel. Hepatic microsomes (1–10 μg) were analyzed by Western blotting. Measurement was done with duplicates of five to six different preparations of microsomes. Values are expressed as means ± SD. ND, not detected.

* Significantly different from control, $P < 0.01$.

† Significantly different from control, $P < 0.05$.

‡ Significantly different from male uremic rats (5 weeks), $P < 0.01$.

of P450 2C11, a typical male-specific form, and 3A2, a male-dominant form, were decreased in male uremic rats. The level of P450 2C6, present in both sexes, was changed little by uremia. In contrast, the level of P450 2A1, abundant in immature rats, was increased. In castrated rats, the levels of P450 2C11 and 3A2 were also decreased. By testosterone treatment of castrated rats, the total content of cytochrome P450, various metabolic activities, and decreased levels of cytochrome P450 2C11 and 3A2 were restored to levels higher than those in control rats. However, these levels were affected only slightly by testosterone treatment of uremic rats. These results indicated that the proportions of cytochrome P450 in hepatic microsomes are not necessarily changed by abnormalities of testosterone in uremic rats. In female uremic rats, the levels of P450 2A1, 3A2, and 2E1 increased, although the level of P450 2C12, a female-specific form, was unchanged. In uremic rats, endocrine factors other than androgens may affect the levels of cytochrome P450.

LeBlanc and Waxman [34] have reported that with the administration of cisplatin (which causes renal failure) to male rats, the serum testosterone level decreases, the P450 2C11 and 3A2 levels decrease, and the P450 2A1 level increases, but that these changes are prevented by the administration of androgen. Their renal failure model and our uremia model cause the changes in the levels of cytochrome P450 by different mechanisms. Thummel and Schenkman [35] have reported that in male rats with diabetes caused by streptozotocin, the serum testosterone level is decreased, P450 2C11 and 3A2 levels are decreased, and P450 2A1 and 2E1 levels are increased, but that by the administration of testosterone, the P450 3A2 level is restored while the levels of the other forms are not changed; treatment with insulin causes the content of cytochrome P450 to return to normal levels. They have suggested that there are factors other than androgen that affect the content of cytochrome P450 in diabetic rats. Adult rats are sexually differentiated with respect to the pattern of pituitary growth hormone (GH) secretion. Adult male rats secrete GH in an episodic or pulsatile way and adult female rats secrete it in a more continuous way [36]. Some forms of cytochrome P450 are regulated by the secretion of GH; P450 2C11 is dependent on the pulsatile secretion of GH [37]; P450 3A2 is suppressively regulated by GH [38]; P450 2A1 seems to be regulated by GH and thyroid hormone [39, 40]. However, details about GH secretion in uremia have not been studied. More studies must be made on the effects of factors (including GH) other than androgen on cytochrome P450 in uremic rats. Also, various uremic toxins appear in the blood in uremia, and this may affect the induction and suppression of the expression of hepatic microsomal cytochrome P450.

In humans, it is not clear whether there is a sex difference in hepatic microsomal cytochrome P450 activity and whether any hormone regulates this activity. Chronic renal failure is associated with gonadal dysfunction in both men and women. In men, uremic hypogonadism is manifested by hypoandrogenism (gynecomastia and loss of libido and potency) and hypospermatogenesis (decreased testicular size, spermatogenesis, and infertility) [41–43]. The features of hypogonadism and infertility have been found in models of uremia in rats. Handelsman [42] has reported that the hypogonadism could be due to direct effects of uremia on the testes or to indirect effects via the reduction in the level of circulating luteinizing hormone (LH) level. Further investigation is needed to identify the cause of the alteration in hepatic drug metabolism in uremic rats.

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