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Induction and regulation of cytochrome *P*450 K-5 (lauric acid hydroxylase) in rat renal microsomes by starvation

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The effects of starvation on rat renal cytochrome *P*-450s were studied. The content of spectrally measured cytochrome *P*-450 in the renal microsomes of male rats increased 2-fold with 72 h starvation, but cytochrome b_5 and NADPH-cytochrome *P*-450 reductase were not induced. 7-Ethoxycoumarin *O*-dealkylation and aniline hydroxylation activities of the renal microsomes of control male rats were very low but were induced 2.5–3-fold by 72 h starvation. Aminopyrine *N*-demethylation and lauric acid hydroxylation activities were induced 1.5–2-fold by 72 h starvation. The changes in catalytic activities suggested that the contents of individual cytochrome *P*-450s in the renal microsomes were altered by starvation. The contents of some cytochrome *P*-450s were measured by Western blotting. *P*450 DM (P450IIE1), a typical form of cytochrome *P*-450 induced by starvation in rat liver, was barely detected in rat kidney and was induced 2-fold by 72 h starvation. *P*450 K-5, a typical renal cytochrome *P*-450 and lauric acid hydroxylase, accounted for 81% of the spectrally measured cytochrome *P*-450 in the renal microsomes of control male rats and was induced 2-fold by 72 h starvation. *P*450 K-5 was not induced in rat kidney by treatment with chemicals such as acetone or clofibrate. The renal microsomes of male rats contained 6-times as much *P*450 K-5 as those of female rats. These results suggest that *P*450 K-5 is regulated by an endocrine factor.

Introduction

Cytochrome *P*-450s are found predominantly in the liver, but also in many other tissues, such as the kidney, and catalyze the oxidative metabolism of many compounds such as drugs and carcinogens as well as endogenous steroids and fatty acids. With the ability to monitor individual cytochrome *P*-450 in physiological conditions, such as starvation, it is possible to find what role an individual cytochrome *P*-450 plays in vivo and how it is regulated. The balance of metabolic activation and detoxification of drugs and other chemicals by individual forms of cytochrome *P*-450 in different tissues is an important factor in organ-specific toxicity. Starvation increases aniline hydroxylation and *N*-nitrosodimethylamine demethylation activities of rat hepatic microsomes [1,2] and also increases the hepatotoxicity of some drugs, such as halogenated hydrocarbon and *N*-nitrosodimethylamine [3,4]. The increase in metabolic activity and hepatotoxicity by starvation arises mainly because of the induction of cytochrome *P*450 DM (P450IIE1) in the liver [5–7]. *P*450 DM is induced in rat liver by ethanol, acetone and diabetes [8–11]. Also, *P*450 DM is suppressively regulated by pituitary growth hormone [12]. Starvation increases the serum level of ketone bodies such as acetone and decreases the level of serum growth hormone [2,13,14]. These two factors affect the increase of *P*450 DM in starvation. In rat liver, cytochrome *P*-450s other than *P*450 DM have also been reported [7,15]. However, little is known about changes in cytochrome *P*-450 in extrahepatic tissues in physiological conditions such as starvation, or about the regulation of cytochrome *P*-450 in extrahepatic tissues.

Starvation increases lauric acid hydroxylation activity of rat hepatic microsomes and the content of cytochrome *P*450 K-5 as well as *P*450 DM in rat liver [15]. *P*450 K-5 has been purified from the renal microsomes of untreated male rats [16]. *P*450 K-5 is different from *P*450 DM and from constitutive hepatic cytochrome *P*-450s, such as *P*450 UT-2 (P450IIC11) and *P*450

Abbreviations: *P*-450, cytochrome *P*-450; HPLC, high-performance liquid chromatography; DEAE, diethylaminoethyl; CM, carboxymethyl; SDS, sodium dodecyl sulfate.

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UT-5 (P450IIC13) [8,17,18]. *P*450 K-5 has high lauric acid hydroxylation activity and low oxidation activity toward drugs, such as benzphetamine and 7-ethoxycoumarin; its molecular weight (52000) is higher than that of *P*450 UT-2 or *P*450 UT-5 (50000 and 48000, respectively) [16,18]. *P*450 K-5 hydroxylates arachidonic acid [19]. An arachidonic acid metabolite generated by cytochrome *P*-450 in cells derived from rabbit kidney inhibits Na^+/K^+-ATPase [20]. It is a possible way in which renal cytochrome *P*-450 has important biological functions, although the significance of the hydroxylation of lauric acid and arachidonic acid is not yet understood. Therefore, it is of interest to find how cytochrome *P*-450 in the kidney is changed in physiological conditions, such as starvation and how cytochrome *P*-450 is regulated.

In this study, we investigated changes in the amount of cytochrome *P*450 K-5 and changes in the catalytic activity of renal microsomes toward some drugs by starvation and also investigated the regulation of *P*450 K-5 in rat renal microsomes.

Materials and Methods

Chemicals

Testosterone, sodium laurate, ω-hydroxylauric acid and dilauroylphosphatidylcholine were obtained from Sigma (St. Louis, MO). NADPH was obtained from Oriental Yeast (Tokyo, Japan). 9-Anthryldiazomethane was obtained from Funakoshi Chemical Industry (Tokyo, Japan). (ω-1)-Hydroxylauric acid (11-hydroxydodecanoic acid) was synthesized by the method of Montellano and Reich [21] as follows. 11-Dodecenoic acid obtained from Nu-Chek-Prep (Elysian, MN) was reacted with mercuric acetate in water and tetrahydrofuran. After alkalinization with NaOH, sodium borohydride was added. Then the mixture was acidified with HCl and was extracted with tetrahydrofuran. The 11-hydroxydodecanoic acid extracted was purified in a silica-gel column. The chemical structure of 11-hydroxydodecanoic acid was confirmed by ^{1}H-NMR. Organic solvents and other reagents including clofibrate and acetone were obtained from Wako Pure Chemical Industries (Tokyo, Japan).

Preparation of microsomes and immunochemical methods

Sprague-Dawley rats weighing 200–250 g were obtained from Charles River (Tokyo, Japan). The animals were starved for 24, 48 or 72 h, with water provided ad libitum. Clofibrate (500 mg/kg) and acetone (500 mg/kg) were given intraperitoneally daily for 3 and 4 days, respectively [22]. The microsomes of the rat kidneys were prepared as described elsewhere [23]. *P*450 K-5 was purified from renal microsomes of untreated male rats as reported previously [16]. Antibody against purified *P*450 K-5 was raised as described previously [24] in a female Japanese white rabbit obtained from Biotech (Saga, Japan). The immunoblotting and immunochemical assay of individual cytochrome *P*-450 isozyme were as reported before [25]. After SDS-polyacrylamide gel electrophoresis with a 7.5% polyacrylamide gel, proteins were transferred from the gel to a nitrocellulose sheet (Bio-Rad Laboratories, Richmond, CA) in a buffer (pH 8.3) containing 100 mM Tris, 192 mM glycine and 20% methanol. The nitrocellulose membrane was treated with antibody and stained with the use of a Vectastain ABC Kit (Vector Laboratories, Burlingame, CA). Immunochemical assays were done by densitometry of the stained nitrocellulose sheets.

Assay methods

Total amounts of cytochrome *P*-450 in microsomes were estimated spectrally by the method of Omura and Sato [26]. The protein concentration was measured by the method of Lowry et al. [27]. 7-Ethoxycoumarin *O*-dealkylation, aminopyrine *N*-demethylation and aniline hydroxylation activities were assayed by methods described elsewhere [18,25]. Lauric acid ω- and (ω − 1)-hydroxylation activities were assayed by conversion of the hydroxylauric acids produced to fluorescent derivatives with 9-anthryldiazomethane and by analysis with reversed-phase HPLC as previously described [28].

Results

Effects of starvation on catalytic activity of renal microsomes

Total cytochrome *P*-450 measured spectrally was induced 2-fold by 72 h starvation, but cytochrome b_5 and

TABLE I

Alterations in the content of total cytochrome P-450, cytochrome b_5 and NADPH-cytochrome P-450 reductase by starvation

The values are expressed as means ± S.D. of five to six different animals. b_5, cytochrome b_5; fp_2, NADPH-cytochrome *P*-450 reductase.

	Content of hemoprotein and reductase			
	control ($n = 5$)	starved (24 h) ($n = 5$)	starved (48 h) ($n = 5$)	starved (72 h) ($n = 6$)
P-450 (nmol/mg)	0.105 ± 0.027	0.144 ± 0.025 *	0.174 ± 0.027 *	0.207 ± 0.036 *
b_5 (nmol/mg)	0.049 ± 0.010	0.047 ± 0.005	0.044 ± 0.007	0.053 ± 0.004
fp_2 (unit/mg)	0.034 ± 0.0058	0.033 ± 0.0040	0.028 ± 0.0049	0.031 ± 0.0095

* Significantly different from control at $P < 0.05$.

NADPH-cytochrome *P*-450 reductase were not induced (Table I). In rat liver, starvation affected the total amount of cytochrome *P*-450 little (1.3-fold induction with 72 h starvation); cytochrome b_5 and NADPH-cytochrome *P*-450 reductase increase little (1.3-fold) [15]. Starvation affected the liver and kidney differently. The effects of starvation on the catalytic activities of renal microsomes are shown in Table II. The aminopyrine *N*-demethylation, 7-ethoxycoumarin *O*-dealkylation and aniline hydroxylation activities of the renal microsomes of control rats were much lower than those of the hepatic microsomes of these rats (1/12, 1/88 and 1/9, respectively), although the specific content of cytochrome *P*-450 in the renal microsomes of these rats is one-sixth that in the hepatic microsomes [15,23]. The lauric acid ω- and (ω-1)-hydroxylation activities of the renal microsomes of control rats per nmol of cytochrome *P*-450 (7.92 and 2.33 nmol/min per nmol of cytochrome *P*-450, respectively) were larger than those of hepatic microsomes (1.73 and 1.73). The renal microsomes used lauric acid as a substrate more readily than drugs such as aniline and 7-ethoxycoumarin. All catalytic activities of renal microsomes tested in this study were increased by starvation. Lauric acid ω-hydroxylation activity was induced 1.5-fold by 72 h starvation. The induction of the lauric acid hydoxylation activity of rat renal microsomes by starvation is in agreement with an earlier report [29]. 7-Ethoxycoumarin *O*-dealkylation and aniline hydroxylation activities were both induced 2.5–3-fold by 72 h starvation. The increase in the aminopyrine *N*-demethylation activity was less than that in the 7-ethoxycoumarin or aniline oxidation activity.

Alterations in the content of P450 K-5 by starvation

A specific antibody was prepared against *P*450 K-5 and its specificity was tested by immunoblotting (Fig. 1A). Anti-*P*450 K-5 antibody did not cross-react with 11 hepatic cytochrome *P*-450s purified previously [11,18,30,31]. Anti-*P*450 K-5 antibody also did not cross-react with two minor renal cytochrome *P*-450s, *P*450 K-2 and K-4 purified previously [32]. Fig. 1B shows Western blots of the renal microsomes of control male rats and male rats starved for 24, 48 or 72 h

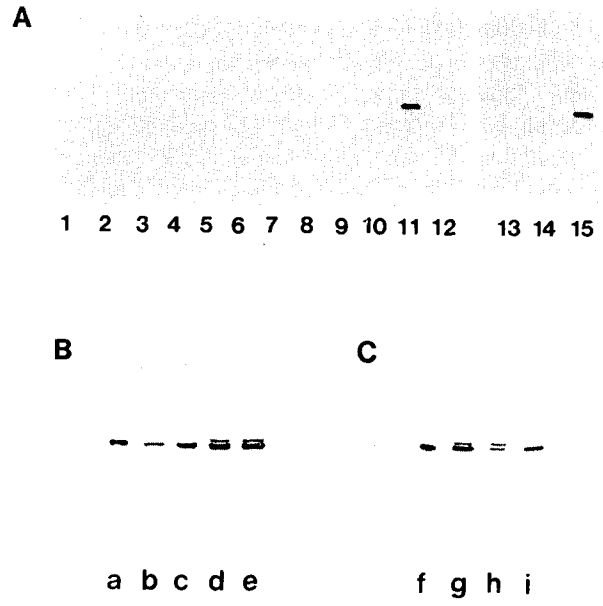

Fig. 1. Western blot analysis of purified cytochrome *P*-450 and renal microsomes from control and starved rats. Lanes 1–15 contain 0.7 pmol of purified cytochrome *P*450 UT-1, UT-2 (P450IIC11), UT-4 (P450IIA2), UT-5 (P450IIC13), PB-1 (P450IIIA2), PB-2 (P450IIC6), PB-4 (P450IIB1), PB-5 (P450IIB2), MC-1 (P450IA2), MC-5 (P450IA1), K-5, DM (P450IIE1), K-2, K-4 and K-5, respectively [11,16,30,32]. The designations given to the rat cytochrome *P*-450s described in this study can be related to the standardized gene designations [39,40]. Lanes a and f contain 0.7 pmol of purified *P*450 K-5. Lanes b, c, d and e contain 5 μg of renal microsomes of male rats starved for 0, 24, 48 and 72 h, respectively. Lanes g, h and i contain 10 μg of renal microsomes of control male rats, control female rats and male rats treated with clofibrate, respectively. Lanes g–i contain 2-times as much proteins as lanes b–e for detection of *P*450 K-5 in the renal microsomes of female rats. Lanes d, e, g and h contain two bands. The band of lower molecular weight corresponds to *P*450 K-5. The band of higher molecular weight may be *P*450 DM-2 as mentioned in the Results. Purified cytochrome *P*-450 and renal microsomes were electrophoresed in 7.5% polyacrylamide gels and transferred electrophoretically to a nitrocellulose membrane. The nitrocellulose was stained immunochemically with anti-*P*450 K-5 antibody.

TABLE II

Alterations of the catalytic activity of rat renal microsomes by starvation

Assay was done with duplicates and the values are expressed as means ± S.D. of five to six different animals.

Substrate	Catalytic activity (nmol/min per mg)			
	control ($n = 5$)	starved (24 h) ($n = 5$)	starved (48 h) ($n = 5$)	starved (72 h) ($n = 6$)
Aminopyrine	0.199 ± 0.057	0.229 ± 0.078	0.285 ± 0.048 *	0.358 ± 0.085 *
7-Ethoxycoumarin	0.017 ± 0.002	0.021 ± 0.003 *	0.033 ± 0.011 *	0.041 ± 0.009 *
Aniline	0.031 ± 0.008	0.066 ± 0.011 *	0.085 ± 0.014 *	0.088 ± 0.016 *
Lauric acid				
(ω − 1)-hydroxylation	0.245 ± 0.023	0.241 ± 0.047	0.274 ± 0.049	0.303 ± 0.082
ω-hydroxylation	0.832 ± 0.107	0.846 ± 0.195	1.10 ± 0.125 *	1.26 ± 0.232 *

* Significantly different from control at $P < 0.05$.

stained immunochemically with anti-*P*450 K-5 antibody. *P*450 K-5 was induced by starvation. An immunochemically stained band not of *P*450 K-5 but from renal microsomes of rats starved for 48 or 72 h (lanes d and e in Fig. 1B) was less mobile than *P*450 K-5 on SDS-polyacrylamide gel electrophoresis. The mobility of this band agreed with that of *P*450 DM-2. *P*450 DM-2 is induced by diabetes in rat liver and has high lauric acid hydroxylation activity and the same NH_2-terminal sequence as *P*450 K-5 [28]. Also, *P*450 DM-2 and K-5 give very similar peptide fragment patterns when digested with α-chymotrypsin and have the same immunochemical reactivity when tested with anti-*P*450 K-5 antibody [28]. However, the molecular weight of *P*450 DM-2 was slightly larger than that of *P*450 K-5. The relationship of *P*450 K-5 and DM-2 may be similar to that of *P*-450b (P450IIB1) and *P*-450e (P450IIB2), which have only 14 differences in their amino acid sequences but have different mobility in SDS-polyacrylamide gel electrophoresis [18,33]. Therefore, the immunostained band above *P*450 K-5 in Fig. 1B may be *P*450 DM-2 induced with starvation in rat kidney. Fig. 2 shows alterations in *P*450 K-5 assayed with Western blots by densitometry of nitrocellulose membranes. Known amounts of purified *P*450 K-5 were used on a nitrocellulose membrane to construct a standard curve. *P*450 K-5 accounted for 81% of the spectrally measured cytochrome *P*-450 in the renal microsomes of control male rats (calculated from the values in Tables I and III or Fig. 2) and was the major renal cytochrome *P*-450. *P*450 K-5 was increased time-dependently by starvation and induced 2-fold by 72 h starvation. In rat liver, *P*450 K-5 was a very minor form (accounted for 1.3% of spectrally measured cytochrome

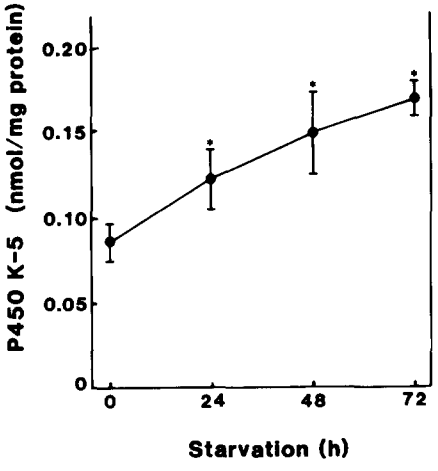

Fig. 2 Alterations in the content of *P*450 K-5 in renal microsomes by starvation. Levels of *P*450 K-5 were assayed by densitometry of a nitrocellulose membrane immunoblotted from an SDS-polyacrylamide gel. Renal microsomes of male rats (5 μg of protein) were analyzed by Western blotting. Measurement was done with duplicates of five or six different animals. * Significantly different from control at $P < 0.05$.

TABLE III

The contents of P450 K-5 and DM in rat renal microsomes

Levels of cytochrome *P*-450s were assayed by densitometry of nitrocellulose immunoblotted from an SDS-polyacrylamide gel. Renal microsomes (5 or 20 μg) were analyzed by Western blotting. Measurement was done with duplicates. The values are expressed as means ± S.D. of five to six different animals in pmol of cytochrome *P*-450 per mg of microsomal protein. The values in parentheses are the contents of *P*450 K-5 or DM in rat hepatic microsomes for comparison. n.d., not decided.

Renal microsomes	*P*450 K-5	*P*450 DM
Untreated male, $n = 5$	84.8 ± 10.7	0.2 ± 0.04
	(8.1 ± 2.3)	(78.8 ± 6.6)
Starved male (72 h), $n = 6$	169.0 ± 10.6 *	0.4 ± 0.11 *
	(17.1 ± 6.2 *)	(162.9 ± 26.5 *)
Acetone-treated male, $n = 5$	74.5 ± 22.8	0.6 ± 0.14 *
	(7.0 ± 1.5)	(153.0 ± 16.3 *)
Clofibrate-treated male, $n = 5$	59.0 ± 10.7 *	n.d.
	(28.8 ± 1.0 *)	
Untreated female, $n = 5$	13.7 ± 0.50 *	n.d.
	(1.7 ± 0.74 *)	

* Significantly different from control at $P < 0.05$.

P-450) [15]. As in renal microsomes, *P*450 K-5 was induced 2-fold in rat liver by 72 h starvation [15].

Regulation of cytochrome P450 K-5 in rat renal microsomes

Starvation induces *P*450 K-5 as well as *P*450 DM in rat liver [15]. Both *P*450 K-5 and *P*450 DM were also induced in rat renal microsomes by starvation (Table III). *P*450 DM, acetone-inducible form in rat liver [9], was induced in rat kidney 3-fold by acetone (Table III). However, acetone did not induce *P*450 K-5. *P*450 K-5 and *P*450 DM seem to be regulated differently. In rat kidney, clofibrate, a strong inducer of lauric acid hydroxylase, did not induce *P*450 K-5, which has high lauric acid hydroxylation activity, although *P*450 K-5 was induced 3-fold by clofibrate in rat liver (Fig. 1C and Table III). The content of *P*450 K-5 in renal microsomes of female rats was much lower than that in male rats (Fig. 1C and Table III). *P*450 K-5 may be regulated by an endocrine factor. In rat liver, the content of *P*450 K-5 in female rats was also one-fifth that in male rats (Table III).

Discussion

A certain form of cytochrome *P*-450 seems to be induced in renal microsomes by starvation, to judge from alterations in its catalytic activity [29]. However, it has not been known which form was induced. *P*450 DM, a typical starvation-inducible form in rat liver, was barely detected in the renal microsomes of control male rats and induced 2-fold by 72 h starvation. The in-

creases in 7-ethoxycoumarin *O*-dealkylation and aniline hydroxylation activities in the renal microsomes of starved rats may be due to an increase in *P*450 DM like that in the rat liver [5–7]. In rat kidneys, *P*450 K-5 was a major cytochrome *P*-450 and was induced with starvation. *P*450 K-5 was detected at a very low level in the liver of control rats and induced with starvation, like *P*450 DM [15].

Starvation increases the level of serum ketone bodies [2,13]. The increase in *P*450 DM is probably due to the increase of ketone bodies such as acetone, because *P*450 DM is induced in rat liver with acetone [9]. *P*450 K-5 was not induced by acetone in either rat liver or kidney. Therefore, *P*450 K-5 and DM are probably regulated differently. The administration of lauric acid to rats increases the total amount of cytochrome *P*-450 in renal microsomes and also increases the lauric acid hydroxylation activity [34]. This phenomenon resembles the induction of cytochrome *P*-450 in rat kidney by starvation. Also, free fatty acids in the serum increase with starvation [13]. Therefore, fatty acids may induce *P*450 K-5 in rat kidneys.

The content of *P*450 K-5 in the renal microsomes of control female rats was much lower than that in control male rats. These results suggest that *P*450 K-5 is regulated by an endocrine factor, just as *P*450 UT-2 (P450IIC11), the male-specific form in rat liver, is regulated by pituitary growth hormone or testosterone [35]. Unlike *P*450 UT-2, *P*450 K-5 was not changed in rat kidney with the reduction of the serum testosterone level by castration (data not shown). Therefore, *P*450 K-5 was a male-dominant form but its regulation in the rat kidney was different from that of *P*450 UT-2 in rat liver. *P*450 K-5 was not induced in the kidney by clofibrate, although this is a strong inducer of lauric acid hydroxylase in the liver [22,36]. Also, *P*450 K-5 was different from *P*-450$_{LA\omega}$ (P450IVA1) by their NH_2-terminal sequences [22]. *P*450 K-5 is induced by starvation and diabetes, as is *P*450 DM, and both forms seem to be regulated by an endocrine factor. However, *P*450 K-5 and *P*450 DM were regulated differently.

The renal cytochrome *P*-450 readily uses fatty acids and prostaglandin as a substrate. Renal cytochrome *P*-450 produces bioactive metabolites from arachidonic acid [20]. The profile of arachidonic acid metabolized by renal cytochrome *P*-450 changes in pathophysiological conditions such as hypertension [20]. Also, ketotic rats and humans excrete considerable amounts of dicarboxylic acids in the urine; these seem to be derived from an endogenous fatty acid by ω-oxidation [37,38]. In ketosis, such as diabetes and starvation, *P*450 K-5 is induced, and probably affects the metabolism of fatty acids. The biological roles of renal cytochrome *P*-450 have not been identified. However, renal cytochrome *P*-450 participates in the metabolism of arachidonic acid and prostaglandin, its biological role may be important. To understand the regulation of renal cytochrome *P*-450 may help to understand its role in vivo.

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