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REGULATION OF RENAL CYTOCHROME P450s BY THYROID HORMONE IN DIABETIC RATS

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Abstract—Effects of thyroid hormone treatment on renal P450 expression in the diabetic rats were investigated. Diabetes produced by streptozotocin induced CYP4A2 and P450 K-2 (similar form with CYP2C23) but not P450 K-4 (similar form with CYP4A8) and induced lauric acid hydroxylation activity. The serum thyroid hormone level was decreased with diabetes. Treatment of diabetic rats with thyroid hormone (T_3) as well as with insulin reversed the increase in the levels of CYP4A2 and P450 K-2. Thyroidectomy also induced CYP4A2 and P450 K-2 in the rat kidney. The increase was reversed by treatment of thyroidectomized rats with T_3. These findings suggest that expression of CYP4A2 and P450 K-2 in rat kidney is suppressively regulated by thyroid hormone and the decrease in thyroid hormone level in the diabetic state affects the levels of CYP4A2 and P450 K-2.

Diabetes is a pathophysiological state that is a useful probe of P450 regulation. Diabetes increases serum glucose and ketone bodies and decreases the serum levels of pituitary growth hormone, androgen, and thyroid hormone [1, 2]. P450 expression is affected by the status of pituitary, gonadal, and thyroidal hormone secretion [3]. Diabetes induces CYP2C7, 2A1, and a 4A form, and especially CYP2E1 in rat livers [4–6]. On the contrary, it suppresses the expression of CYP2C11 and 2C13, male-specific forms [4–6]. CYP2E1 is induced by ketones such as acetone, and its expression is suppressively regulated by growth hormone [7–9]. Increased levels of ketone bodies and a decreased level of pituitary growth hormone seem to induce CYP2E1. However, treatment with testosterone and growth hormone only partly reverses these changes, although treatment of diabetic rats with insulin reverses changes in the levels of these P450s [1, 10]. Insulin treatment reverses many factors such as serum glucose and ketone body levels in diabetic rats. Therefore, which factor mainly contributes to pathophysiological changes in P450s in the diabetic state is not clear.

Rat kidney has the next highest amount of P450 after the livers. Renal P450s have different properties from hepatic P450s. Fatty acids such as lauric acid and arachidonic acid are better substrates for renal P450s than drugs such as aminopyrine and 7-ethoxycoumarin, which are often used for the characterization of hepatic P450s such as CYP2B1 and 1A1 [11], and the induction of P450 by phenobarbital is low in rat kidney [12]. Therefore, the regulation of renal P450 is probably different from that of hepatic P450. Recently, Sundseth and Waxman [13] reported that renal P450 is directly regulated by testosterone. We also obtained similar results [14]. However, the regulation of renal P450 has not been studied as much as that of hepatic P450.

In this study, we investigated the changes in the levels of P450 in rat kidney by diabetes and the effects of thyroid hormone on the regulation of P450 expression in diabetic rats.

MATERIALS AND METHODS

Treatment of animals. Sprague–Dawley rats were obtained from Clea Japan (Tokyo). Diabetes was induced with a single injection of streptozotocin (STZ, 65 mg/kg) intravenously at 7 weeks of age. Insulin (6 IU/rat/day, two injections a day) was given subcutaneously to diabetic rats. Thyroidectomy was done at 7 weeks of age. T_3 (5 or 50 μg/kg) was given subcutaneously to rats. The treatments with insulin or T_3 were initiated at 8 weeks of age and continued for 2 weeks. Rats were killed at 10 weeks of age. Serum glucose was measured by a glucose oxidase method [15]. Serum T_3 was assayed by a radioimmunoassay (RIA) using a SPAC T_3 RIA kit (Daiichi Radioisotope Laboratory, Tokyo).

Immunochemical assay of P450s. P450 K-2, K-4, and CYP4A2 (K-5) were purified from renal microsomes of untreated male rats [16]. The NH_2-terminal amino acid sequences of P450 K-2 and K-4 were similar to those of the deduced amino acid sequences of CYP2C23 [17, 18] and CYP4A8 [19, 20], respectively. Antibodies against purified P450s were prepared with Japanese White rabbits

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(Biotech, Saga, Japan). Characterization of antibodies has been described elsewhere [21]. Each form of P450 was assayed by a quantitative immunoblotting described previously [21]. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was done with 7.5% gels for analysis of microsomal cytochrome P450. Microsomal proteins (5–30 μg) were electrophoresed and electrophoretically transferred to a nitrocellulose membrane. The transferred cytochrome P450 was reacted with a specific antibody from a rabbit and was stained with a Vectastain ABC kit with horseradish peroxidase (Vector Laboratory). Quantitation was done by densitometry of the stained band. The microsomal levels of individual cytochrome P450 forms were estimated relative to purified cytochrome P450 as a standard. Assay of lauric acid hydroxylation activity was described elsewhere [16].

RESULTS

Effects of thyroid hormone on renal P450 in diabetic rats. Induction of diabetes caused by STZ was evident from the elevated blood glucose levels seen in these rats (Table 1). Insulin treatment lowered the blood glucose level. As previously reported [2], diabetes caused by STZ decreased the serum T_3 level. Insulin treatment reversed the decrease in T_3 level. The content of total P450 measured photometrically was increased by diabetes. Lauric acid hydroxylation activity was also induced by diabetes. Changes in the three forms of P450 caused by diabetes in the rat kidney were measured by immunoblotting (Fig. 1 and Table 1). Diabetes did not induce P450 K-4, which is a minor renal form, but induced significantly CYP4A2, a major renal form, and P450 K-2. The increase was reversed by treatment of diabetic rats with insulin. Thummel and Schenkman [1] found that diabetes decreases the levels of serum testosterone and growth hormone and that the changes in the levels of P450 caused by diabetes in rat hepatic microsomes are not recovered

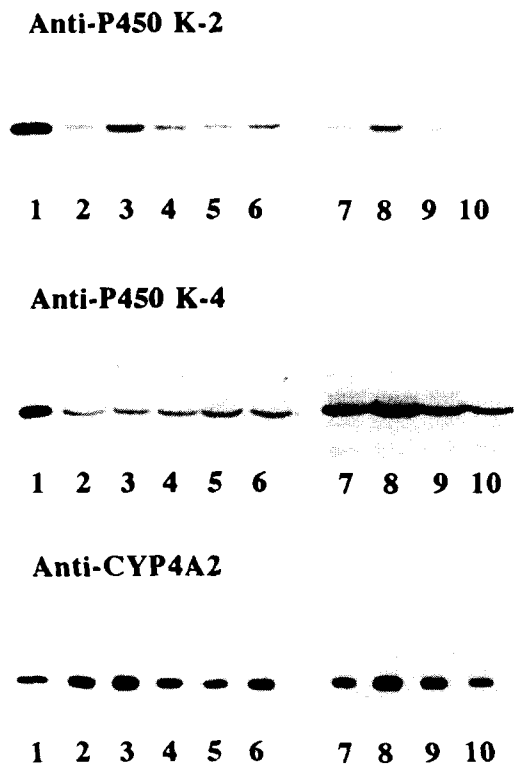

Fig. 1. Immunoblots of renal microsomes from diabetic and thyroidectomized rats. Lane 1 contained a purified P450 (0.5 pmol) with a corresponding antibody shown at the top of the membrane. Each lane contained renal microsomes from control rats (lane 2), diabetic rats (lane 3), diabetic rats treated with low dose T_3 (5 μg/kg, lane 4), treated with high dose T_3 (50 μg/kg, lane 5), treated with insulin (lane 6), sham-operated rats (lane 7), thyroidectomized rats (lane 8), thyroidectomized rats treated with low dose T_3 (5 μg/kg, lane 9), and treated with high dose T_3 (50 μg/kg, lane 10). Each membrane contained the following amounts of microsomal protein in each lane: 15 μg for anti-P450 K-2 antibody, 30 μg for anti-P450 K-4 antibody, and 5 μg for anti-CYP4A2 antibody.

Table 1. Effects of diabetes on levels of rat renal P450s

	Control	DM	DM + insulin	DM + LT_3	DM + HT_3
N	5	7	6	6	6
Glucose (mg/dL)	112 ± 8	487 ± 19*	80 ± 23†	471 ± 24	463 ± 23
T_3 (ng/mL)	0.88 ± 0.08	0.62 ± 0.07*	0.89 ± 0.15†	0.54 ± 0.11	1.15 ± 0.26†
Total P450 (nmol/mg)	0.085 ± 0.011	0.115 ± 0.016*	0.078 ± 0.010†	0.101 ± 0.012	0.080 ± 0.017†
Lauric acid (nmol/min/mg)					
(ω − 1)-Hydroxylation	0.26 ± 0.05	0.56 ± 0.12*	0.32 ± 0.07†	0.46 ± 0.10	0.31 ± 0.07†
ω-Hydroxylation	0.60 ± 0.14	1.09 ± 0.22*	0.74 ± 0.09†	0.90 ± 0.26	0.61 ± 0.18†
P450 forms (pmol/mg)					
K-2	5.2 ± 0.6	7.6 ± 0.5*	6.1 ± 0.5†	5.4 ± 1.3†	5.0 ± 1.0†
K-4	2.3 ± 0.6	2.7 ± 0.6	2.0 ± 0.1	2.3 ± 0.4	1.8 ± 0.5‡
4A2	77.7 ± 7.6	130.1 ± 9.8*	101.4 ± 10.5†	125.2 ± 12.4	66.0 ± 7.7†

The total content of P450 was assayed photometrically. The levels of each form of P450 were measured by immmunoblotting. Values are means ± SD. DM, diabetic rats induced with STZ; DM + insulin, diabetic rats treated with insulin; DM + LT_3, diabetic rats treated with low dose T_3 (5 μg/kg); DM + HT_3, diabetic rats treated with high dose T_3 (50 μg/kg).

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

† Significantly different from diabetic rats, $P < 0.01$.

‡ Significantly different from diabetic rats, $P < 0.05$.

Table 2. Effects of thyroidectomy on levels of rat renal P450s

	Sham	Thydx	Thy + LT_3	Thy + HT_3
N	5	5	4	5
T_3 (ng/mL)	0.98 ± 0.08	0.64 ± 0.13*	0.63 ± 0.11	1.03 ± 0.32§
Total P450 (nmol/mg)	0.075 ± 0.014	0.107 ± 0.026†	0.090 ± 0.019	0.053 ± 0.008‡
Lauric acid (nmol/min/mg)				
(ω − 1)-Hydroxylation	0.36 ± 0.03	0.56 ± 0.16†	0.43 ± 0.09	0.31 ± 0.06§
ω-Hydroxylation	0.74 ± 0.09	1.33 ± 0.38†	0.89 ± 0.22	0.65 ± 0.15§
P450 forms (pmol/mg)				
K-2	5.7 ± 1.7	10.9 ± 0.4*	7.6 ± 2.0§	5.9 ± 1.7‡
K-4	2.0 ± 0.3	2.3 ± 0.3	2.0 ± 0.5	1.4 ± 0.3‡
4A2	84.3 ± 20.1	127.8 ± 8.9*	114.9 ± 24.6	74.8 ± 23.0‡

The total content of P450 was assayed photometrically. The levels of each form of P450 were measured by immmunoblotting. Values are means ± SD. Sham, sham-operated rats; thydx, thyroidectomized rats; Thy + LT_3, thyroidectomized rats treated with low dose T_3 (5 μg/kg); Thy + HT_3, thyroidectomized rats treated with high dose T_3 (50 μg/kg).

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

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

‡ Significantly different from thyroidectomized, $P < 0.01$.

§ Significantly different from thyroidectomized, $P < 0.05$.

by treatment with growth hormone and testosterone although they are recovered completely by insulin treatment. Sundseth and Waxman [13] found that the CYP4A2 mRNA level in rat kidney is not affected by growth hormone treatment and is decreased by the decreased level of serum testosterone. If the decreased levels of testosterone and growth hormone affect the level of renal P450 in the diabetic state, the level of CYP4A2 will be decreased. However, the level of CYP4A2 was increased by diabetes. Other factors affect the CYP4A2 level in diabetic rats. Thus, the thyroid hormone was given to diabetic rats, because diabetes decreases the serum T_3 level as well as testosterone and growth hormone levels [1]. The increase in the levels of CYP4A2 was prevented by treatment of diabetic rats with T_3 dose dependently and the increase in lauric acid hydroxylation activity was also reversed (Table 1). These results suggest that induction of a major renal P450 caused by diabetes was due to the decrease in serum thyroid hormone level. The increased level of P450 K-2 was also reversed by T_3 treatment.

Effects of thyroidectomy on the levels of renal P450. We further investigated the effects of thyroid hormone on the renal P450 by thyroidectomy and hormone replacement study (Fig. 1 and Table 2). Thyroidectomy of male rats significantly decreased T_3 levels and increased the content of total P450 and lauric acid ω- and (ω − 1)-hydroxylation activities, indicating an increase in the levels of P450 forms. In fact, the levels of P450 K-2 and CYP4A2 measured by immunoblotting were increased by thyroidectomy. Treatment of thyroidectomized rats with T_3 prevented the increases in the levels of these forms. These findings suggest that these forms are suppressively regulated by thryoid hormone. The level of P450 K-4 was not affected by diabetes or thyroidectomy. P450 K-4 may belong to the CYP4A subfamily but its regulation was different from that of CYP4A2, because CYP4A2 was induced by diabetes and thyroidectomy.

DISCUSSION

The present study establishes that diabetes caused by STZ induced CYP4A2 and P450 K-2 in the rat kidney but not P450 K-4. CYP4A2 is a major male-predominant form in the rat kidney, and its level is decreased by the decrease in serum level of testosterone or growth hormone [14]. Diabetes decreases the levels of serum testosterone and growth hormone [1] but induced CYP4A2. Expression of CYP2C11, a major male-specific form in rat liver, is suppressed by diabetes [4–6]. The major renal P450 and the major hepatic P450 are regulated differently, although both are male-predominant forms. In rat liver, increased levels of ketone bodies as well as changes in the hormone levels caused by diabetes induced P450s such as CYP2E1 [8, 9, 22]. However, acetone treatment does not induce CYP4A2 in rat kidney [23]. Factors other than acetone, testosterone, and growth hormone regulate the expression of CYP4A2. In this study, we found that thyroid hormone (T_3) as well as insulin reversed the level of CYP4A2 in diabetic rats. Expression of CYP4A2 and P450 K-2 in the rat kidney was suppressively regulated by thyroid hormone. In rat liver, CYP2B1, 2B2 and 2A1 are suppressively regulated by thyroid hormone [24, 25]. The hepatic content of these forms in rat liver is increased by thyroidectomy and the increase is reversed by treatment with thyroid hormone. The response of these forms to the thyroid hormone seems similar to that of CYP4A2 and P450 K-2. A low dose of T_3 (5 μg/kg) reversed the increased level of P450 K-2 but not that of CYP4A2 in diabetic and thyroidectomized rats, although a high dose (50 μg/kg) suppressed expression of both forms. Regulation of CYP4A2 was different from that of P450 K-2. Hypophysectomy decreases the level of CYP4A2 but induces P450 K-2 in rat kidney [14]. Thyroid hormone affects the level of other hormones such as growth hormone [25], and it is possible that expression of these forms was regulated indirectly

by thyroid hormone. The thyroid hormones have catabolic effects on the metabolism of carbohydrates and lipids. P450 in the CYP4A subfamily can metabolize fatty acids [16]. Increased urinary excretion of dicarboxylic acids, which may be derived from ω-oxidation of medium chain fatty acid in the diabetic condition, has been reported [26]. The induction of CYP4A2 may alter the metabolism of fatty acids in diabetes.

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