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# CHANGES IN AMOUNTS OF CYTOCHROME P450 ISOZYMES AND LEVELS OF CATALYTIC ACTIVITIES IN HEPATIC AND RENAL MICROSOMES OF RATS WITH STREPTOZOCIN-INDUCED DIABETES

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**Abstract**—Hepatic microsomal cytochrome P450s, which are involved in the metabolism of drugs, hormones, prostaglandins and fatty acids, change when animals develop diabetes. We studied changes in cytochrome P450 isozymes in both hepatic and renal microsomes of rats with diabetes caused by streptozocin, and compared the results with changes in catalytic activities in the microsomes. In hepatic microsomes of diabetic rats, the amount of cytochrome P450 2E1, an acetone-inducible isozyme, was two and a half times that of control rats, and that of P450 4A2, a major renal isozyme, was three times that in the controls. The amounts of cytochrome P450s 2A1, 2C6, 2C7, 3A2 and 4A3 increased in hepatic microsomes of diabetic rats, and P450 2C11 decreased. Treatment with insulin restored these to the levels in the controls. The catalytic activities of aniline hydroxylation, 7-ethoxycoumarin O-dealkylation, testosterone 2$\beta$, 6$\beta$, 7$\alpha$, and 16$\beta$-hydroxylation, and $\omega$-, ($\omega$ − 1)-hydroxylation of lauric acid were high in the hepatic microsomes of diabetic rats, and testosterone 2$\alpha$ and 16$\alpha$-hydroxylation activities were low. In renal microsomes of diabetic rats, cytochrome P450s 2E1, 4A2 and K-4 were induced, and $\omega$- and ($\omega$ − 1)-hydroxylation activities were high. These changes were reversed by insulin treatment. The induction and suppression of cytochrome P450 isozymes in diabetic rats were consistent with the changes in the catalytic activities. In both hepatic and renal microsomes, P450s 2E1 and 4A2 were induced, altered metabolism of ketones and fatty acids in diabetes may contribute to these changes.

Cytochrome P450s catalyse the oxidative metabolism of a variety of substrates: some are xenobiotics, including substances that occur biologically but are foreign to animals, such as antibiotics and unusual compounds from plants, and synthetic organic chemicals like steroids and other physiologically occurring lipids [1–3]. Cytochrome P450 isozymes may be induced or suppressed in pathological conditions such as diabetes and hypertension [4, 5]. Rats with diabetes induced by a diabetogenic chemical like streptozocin and spontaneously diabetic rats have changes in the amounts of cytochrome P450s of hepatic microsomes [6, 7]. Diabetes increases the aniline hydroxylation and *N*-nitrosodiomethylamine demethylation activity of hepatic microsomes in rats [7, 8], indicating that some P450 isozymes are induced. The major diabetes-inducible cytochrome P450 seems to be P450 2E1 (P450j), which is the isozyme induced by acetone, ethanol or starvation [9–11]. Another cytochrome P450 isozyme is induced in hepatic microsomes of diabetic rats [12]. It resembles the major renal cytochrome P450, P450 4A2, and recently the isozyme was designated P450 4A3 [13]. Favreau and Schenkman [14] observed the induction of P450 2C7 as well as P450 2E1 and the suppression of P450 2C11 and 2C13 in the liver of diabetic rats. However, changes in the amounts of individual cytochrome P450 isozymes in diabetic rat kidneys have not been fully investigated.

This study examined changes in cytochrome P450 isozymes and catalytic activities of hepatic and renal microsomes in diabetic rats. Diabetes was induced in male rats with streptozocin and some diabetic rats were treated with insulin. Here, specific polyclonal antibodies to cytochrome P450s were prepared. These antibodies were used to identify the amounts of the cytochrome P450 isozymes. Cytochromes P450 2A1, 2C6, 2C7, 2C11, 2E1, 3A2, 4A2 and 4A3 were measured in hepatic microsomes, and cytochrome P450 2E1, K-2, K-4, 4A1 and 4A2 were measured in renal microsomes of these diabetic rats.

## MATERIALS AND METHODS

*Animals and treatment.* Diabetes was induced in male, 7-week old, Sprague–Dawley rats, weighing 220–250 g with an intravenous injection of 65 mg/kg body weight streptozocin (the Sigma Chemical Co., St Louis, MO, U.S.A.). In some diabetic rats, insulin treatment (2 U of isophane insulin at 9.00 a.m. and 4 U at 8.00 p.m.) was started 2 weeks after the streptozocin injection. Rats were killed by decapitation 2, 3 or 5 weeks after the streptozocin injection. The liver and kidneys were removed and perfused with iced 1.15% (w/v) KCl solution containing 1 mM EDTA and 0.25 mM phenylmethylsulfonyl fluoride (Sigma). Hepatic and renal

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microsomes were prepared as described elsewhere [15, 16]. Tissues were homogenized with a Teflon homogenizer in 3 vol. of the same KCl solution as for tissue perfusion, and the homogenates were centrifuged. The isolated pellets were washed with the same solution. The microsomal pellet was suspended in 0.1 M potassium phosphate buffer (pH 7.4), 1 mM EDTA, 1 mM dithiothreitol (Sigma), and 30% (v/v) glycerol to a concentration of 30–50 mg of protein/mL and stored at −90°.

*Assays of cytochrome P450s.* The total amount of cytochrome P450 in microsomes was measured spectrally by the method of Omura and Sato [17]. The protein concentration was measured by the method of Lowry *et al.* [18]. The concentrations of cytochrome P450 isozymes were assayed by immunoblotting [11, 19]. Microsomal samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis with a 7.5% polyacrylamide gel. Microsomal proteins were transferred from the gel to a nitrocellulose sheet (Bio-Rad Laboratories, Richmond, CA, U.S.A.) in 100 mM Tris buffer (pH 8.3) containing 192 mM glycine and 20% (v/v) methanol. The nitrocellulose membrane was treated with an antibody and stained with use of a Vectastain ABC kit® (Vector Laboratories, Burlingame, CA, U.S.A.). Antibodies against P450 isozymes were raised in female Japanese White rabbits as previously described [20]. The characterization of the antibodies against P450 2A1, 2C6, 2C11, 2E1, 3A2 and 4A2 was reported elsewhere [11]. Cytochrome P450 2C7 was purified by the method used in the purification of P450 3A2 [21]. Anti-P450 2C6, 2C7, 2C11, 4A1 and 4A2 antibodies were purified by affinity chromatography [20]. Individual cytochrome P450 isozymes were assayed by densitometry of the stained nitrocellulose sheets with purified P450s as standards.

*Catabolic activity.* Each reaction mixture, in a final volume of 0.5 mL, contained microsomes (200 μg of protein), 0.2 μmol of NADPH, and a substrate. All assays were done with shaking at 37°. Aniline hydroxylation activity was assayed by measurement of the formation of *p*-aminophenol from aniline by colorimetry [22]. The O-dealkylation activity of 7-ethoxycoumarin (Aldrich Chemicals, Milwaukee, WI, U.S.A.) was measured by the method of Guengerich [23]. Testosterone hydroxylation activity was measured by a method described before [21]. Hepatic or renal microsomes were incubated with 0.2 μmol of NADPH and 0.5 μmol of testosterone (Sigma). Testosterone metabolites were extracted and analysed by HPLC with a reverse-phase column (4.0 × 150 mm, RP-18T, Irica, Kyoto, Japan). The column was developed with a linear gradient of a mixture of $H_2O$, $CH_3OH$, and $CH_3CN$ (62:36:2 to 30:64:6) for 20 min. The chromatography was done at a flow rate of 0.7 mL/min at the column temperature of 45°, and the metabolites were monitored at 254 nm. Lauric acid ω- and (ω − 1)-hydroxylation activities were assayed by conversion of the hydroxylauric acids produced to fluorescent derivatives and by analysis with HPLC [24]. After incubation of microsomes with 0.2 μmol of NADPH and 0.1 μmol of sodium laurate (Sigma), the metabolites were extracted with ethyl acetate and converted to their fluorescent ester derivatives with 9-anthryldiazomethane (Funakoshi Co., Ltd, Tokyo, Japan). The ω- and (ω − 1)-hydroxylation acid ester derivatives were separated by HPLC with a reverse-phase column (ODS-120A, Tosoh Corp., Tokyo, Japan) and eluted with a mixture of $CH_3CH$ and $H_2O$ (78:22) at the flow rate of 1.2 mL/min at 40°. The amount of the metabolites was measured by comparison of their peak areas monitored by the fluorescent intensity at 412 nm with excitation at 365 nm.

*Inhibition by anti-P450 antibodies.* The hepatic or renal microsomes (100 μg of protein) of diabetic rats were incubated with different concentrations of anti-P450 antibodies at room temperature for 30 min. The catalytic activities of ω- and (ω − 1)-hydroxylation of lauric acid were measured. Results were expressed as relative activities, which were calculated as the metabolic activity with an antibody divided by that with control immunoglobulin G (IgG*).

*Other assays.* Serum glucose was measured by a glucose oxidase method [25]. Concentrations of 3-hydroxybutyric acid (3-OHBA) were measured by a test-strip method with a reflectance meter [26].

The results were expressed as means ± SE. Statistical analysis was done with the unpaired Student's *t*-test.

## RESULTS

The concentrations of serum glucose and 3-OHBA in diabetic rats are shown in Table 1. The administration of streptozocin raised the glucose concentration, which stayed higher for the 5 weeks of the study period. Treatment with insulin starting 2 weeks after the streptozocin injection, decreased the glucose concentrations to the levels of control rats. The concentrations of 3-OHBA had significantly increased by 2 weeks after the streptozocin injection, and the concentrations were still higher at 5 weeks. At week 3, insulin treatment (for 1 week) did not affect 3-OHBA, but at week 5, the treatment (for 3 weeks) decreased the 3-OHBA level.

The total amount of P450 in hepatic microsomes of diabetic rats was significantly higher than that of control rats (Table 2). At week 3, treatment of diabetic rats with insulin (for 1 week) caused a significant decrease in total P450, but the decrease was insignificant in diabetic rats treated with insulin for 3 weeks. The amounts of cytochrome P450s 2A1, 2C6, 2C7, 2E1, 3A2, 4A2 and 4A3 in hepatic microsomes of diabetic rats were higher than those of control rats. The increase was almost prevented by insulin treatment. Hepatic P450 2E1 in diabetic rats 2 weeks after the streptozocin injection was 2.5-fold that of control rats, and it was suppressed by insulin treatment for 1 or 3 weeks. Diabetic rats had a 2.2-fold higher amount of cytochrome P450 2A1 2 weeks after the streptozocin injection than control rats. Hepatic P450 4A2 was three times higher than that of control rats. The amount of hepatic P450 2C11 in diabetic rats was 60% less than that in control rats, and insulin treatment partly prevented

* Abbreviations: IgG, immunoglobulin G; 3-OHBA, 3-hydroxybutyric acid.

Table 1. Concentrations of serum glucose and 3-OHBA in control rats, diabetic rats without treatment and diabetic rats treated with insulin

| | | Diabetes | | | | Diabetes plus insulin* | |
|---|---|---|---|---|---|---|---|
| | Control | 1 week | 2 week | 3 week | 5 week | 3 week | 5 week |
| Glucose (mM) | 7.7 ± 0.2 | 28.8 ± 1.0† | 27.1 ± 0.7† | 25.4 ± 2.0† | 25.8 ± 2.2† | 4.4 ± 0.5‡ | 3.8 ± 0.1‡ |
| 3-OHBA (μM) | 92 ± 5 | 101 ± 47 | 348 ± 28† | 526 ± 70† | 719 ± 34† | 546 ± 119 | 77 ± 17‡ |

Weeks shown are the numbers of weeks after the streptozocin injection.
Values are means ± SE for six rats.
* Insulin treatment was started 2 weeks after the streptozocin injection.
† $P < 0.001$ compared with control rats.
‡ $P < 0.001$ compared with rats with diabetes of the same duration but without treatment.

Table 2. Changes in total P450 and cytochrome P450 isozymes of hepatic microsomes

| | | Diabetes | | | Diabetes plus insulin | |
|---|---|---|---|---|---|---|
| | Control | 2 week | 3 week | 5 week | 3 week | 5 week |
| Total P450 | 0.50 ± 0.07 | 0.76 ± 0.04‡ | 0.84 ± 0.02§ | 0.79 ± 0.05‡ | 0.71 ± 0.02§ | 0.68 ± 0.05 |
| P450 | | | | | | |
| 2A1 | 5.9 ± 1.0 | 12.9 ± 1.2‡ | 11.9 ± 1.6‡ | 18.6 ± 1.4§ | 5.5 ± 0.8‡ | 5.3 ± 0.2§ |
| 2C6 | 64.7 ± 14.3 | 97.3 ± 16.0 | 111.0 ± 6.0† | 108.0 ± 14.5 | 78.7 ± 10.9* | 78.0 ± 11.0 |
| 2C7 | 25.8 ± 3.3 | 51.1 ± 7.7† | 39.2 ± 4.9* | 36.9 ± 2.1† | 20.0 ± 2.3‡ | 16.8 ± 5.8‡ |
| 2C11 | 404.0 ± 68.6 | 162.0 ± 28.6‡ | 157.0 ± 66.7* | 236.0 ± 22.9* | 218.0 ± 58.0 | 406.7 ± 85.7 |
| 2E1 | 54.0 ± 5.9 | 134.0 ± 29.3* | 128.0 ± 13.2§ | 143.0 ± 23.5‡ | 74.0 ± 6.5‡ | 68.8 ± 4.7† |
| 3A2 | 77.0 ± 4.9 | 116.2 ± 19.6 | 159.3 ± 24.9‡ | 158.6 ± 13.0§ | 106.4 ± 10.5 | 88.9 ± 17.2‡ |
| 4A1 | 16.9 ± 1.3 | 14.3 ± 1.3 | 15.7 ± 1.3 | 13.3 ± 1.2 | 18.1 ± 3.0 | 16.4 ± 0.8 |
| 4A2 | 14.8 ± 1.7 | 43.7 ± 3.1§ | 48.5 ± 4.8§ | 55.5 ± 5.4§ | 21.5 ± 3.2§ | 33.4 ± 3.8‡ |
| 4A3 | 26.0 ± 1.4 | 64.0 ± 3.8§ | 64.0 ± 5.1§ | 69.0 ± 8.9§ | 37.5 ± 4.2‡ | 51.4 ± 3.7 |

Values are expressed as means ± SE in nmol (total P450) or pmol (isozymes) of P450/mg of microsomal protein (N = 6 in each group).
* $P < 0.05$, † $P < 0.02$, ‡ $P < 0.01$ and § $P < 0.001$, diabetic rats vs control rats, or insulin-treated rats vs diabetic rats without treatment.

Table 3. Changes in catalytic activities of hepatic microsomes

| | | Diabetes | | | Diabetes plus insulin | |
|---|---|---|---|---|---|---|
| | Control | 2 week | 3 week | 5 week | 3 week | 5 week |
| Aniline | 0.63 ± 0.03 | 1.05 ± 0.06‡ | 1.17 ± 0.03‡ | 1.07 ± 0.10† | 0.70 ± 0.01‡ | 0.76 ± 0.01* |
| 7-Ethoxycoumarin | 2.05 ± 0.17 | 4.64 ± 0.20‡ | 4.65 ± 0.28‡ | 4.78 ± 0.50‡ | 3.83 ± 0.28 | 4.14 ± 0.26 |
| Testosterone | | | | | | |
| 2α | 1.26 ± 0.07 | 0.57 ± 0.09‡ | 0.70 ± 0.07‡ | 0.59 ± 0.07‡ | 1.22 ± 0.11† | 1.35 ± 0.16† |
| 2β | 0.16 ± 0.01 | 0.45 ± 0.04‡ | 0.50 ± 0.04‡ | 0.44 ± 0.03‡ | 0.26 ± 0.01‡ | 0.30 ± 0.02† |
| 6β | 1.02 ± 0.13 | 2.88 ± 0.29‡ | 2.87 ± 0.31‡ | 2.91 ± 0.10‡ | 1.69 ± 0.11† | 1.69 ± 0.16‡ |
| 7α | 0.15 ± 0.01 | 0.33 ± 0.03‡ | 0.26 ± 0.01‡ | 0.30 ± 0.02‡ | 0.16 ± 0.01‡ | 0.20 ± 0.01† |
| 16α | 1.99 ± 0.11 | 1.00 ± 0.10‡ | 1.39 ± 0.14† | 0.93 ± 0.10‡ | 2.17 ± 0.23* | 2.22 ± 0.26‡ |
| 16β | 0.12 ± 0.01 | 0.23 ± 0.01‡ | 0.22 ± 0.02† | 0.20 ± 0.02† | 0.15 ± 0.01* | 0.16 ± 0.01 |
| Lauric acid | | | | | | |
| ω | 0.28 ± 0.03 | 1.16 ± 0.11‡ | 1.18 ± 0.10‡ | 1.06 ± 0.23† | 0.47 ± 0.04‡ | 0.73 ± 0.07 |
| ω − 1 | 0.43 ± 0.03 | 1.17 ± 0.06‡ | 1.11 ± 0.08‡ | 0.85 ± 0.10† | 0.51 ± 0.02‡ | 0.66 ± 0.03 |

The catalytic activities in six different preparations of hepatic microsomes were assayed in duplicate, and the values are expressed as means ± SE in nmol of product/min/mg of protein.
* $P < 0.02$, † $P < 0.01$ and ‡ $P < 0.001$, diabetic rats vs control rats, or insulin-treated rats vs diabetic rats without treatment.

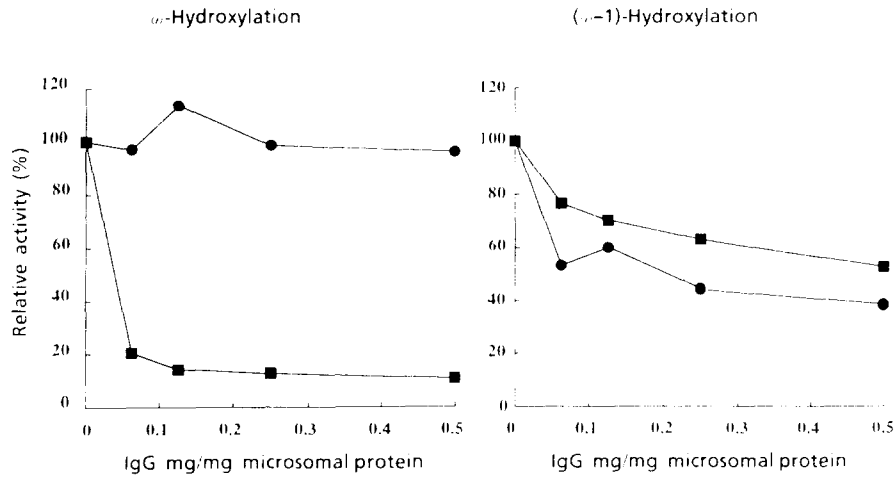


Fig. 1. Effects of anti-P450 2E1 antibody (●) and anti-P450 4A2 antibody (■) on ω- and (ω − 1)-hydroxylation activities of lauric acid in hepatic microsomes of diabetic rats.

it. The amount of cytochrome P450 4A1 in diabetic rats was not different from that in control rats.

The catalytic activities of hepatic microsomes in diabetic rats are shown in Table 3. The activities of aniline hydroxylation, 7-ethoxycoumarin O-dealkylation, testosterone 2β-, 6β-, 7α-, and 16β-hydroxylation, and ω- and (ω − 1)-hydroxylation of lauric acid were significantly higher in diabetic rats 2, 3 and 5 weeks after the streptozocin injection than in control rats. Diabetic rats treated with insulin had lower catalytic activities of these substrates than diabetic rats without the treatment, but 7-ethoxycoumarin O-dealkylation activities in rats treated with insulin did not change significantly. The activities of testosterone 2α- and 16α-hydroxylation of hepatic microsomes in diabetic rats were significantly lower than those in control rats. Insulin treatment for 1 or 3 weeks caused increases in these hydroxylation activities.

Figure 1 shows the effects of anti-P450 2E1 and 4A2 antibodies on ω- and (ω − 1)-hydroxylation activities of lauric acid in hepatic microsomes. The activity of ω-hydroxylation of lauric acid in hepatic microsomes incubated with anti-P450 4A2 antibody was only 5% of the activity with control IgG. Both antibodies against cytochrome P450 4A2 and 2E1 caused decreases in the (ω − 1)-hydroxylation activity of lauric acid in hepatic microsomes. The (ω − 1)-hydroxylation activity was suppressed by 50% with anti-P450 2E1 antibody and suppressed by 40% with anti-P450 4A2 antibody.

The amount of total P450 in renal microsomes of diabetic rats was less than that in hepatic microsomes. The amount was not significantly different from that in control rats (Table 4). Cytochrome P450 2E1 was detected in renal microsomes at low concentrations, and diabetic rats had higher P450 2E1 in renal microsomes than control rats. P450 K-2 was induced in renal microsomes of the diabetic rats. These changes were prevented by treatment with insulin. The major isozyme in renal microsomes was P450 4A2, which was significantly more abundant in diabetic rats than in control rats. Insulin treatment for 1 week reduced the induction of P450 4A2 in

Table 4. Changes in total P450 and cytochrome P450 isozymes of renal microsomes

| | | Diabetes | | | Diabetes plus insulin | |
|---|---|---|---|---|---|---|
| | Control | 2 week | 3 week | 5 week | 3 week | 5 week |
| Total P450 | 93 ± 11 | 121 ± 16 | 106 ± 8 | 77 ± 13 | 78 ± 4 | 96 ± 15 |
| P450 | | | | | | |
| 2E1 | 0.7 ± 0.1 | 1.4 ± 0.2‡ | 4.4 ± 0.4§ | 3.0 ± 0.3§ | 0.9 ± 0.2§ | 0.9 ± 0.2§ |
| K-2 | 6.7 ± 0.6 | 9.3 ± 1.0* | 9.4 ± 1.8 | 13.4 ± 1.6‡ | 6.1 ± 0.5 | 6.6 ± 0.5‡ |
| K-4 | 1.8 ± 0.6 | 2.7 ± 0.5 | 2.7 ± 0.3 | 1.9 ± 0.3 | 1.6 ± 0.1 | 1.6 ± 0.2 |
| 4A1 | 0.3 ± 0.1 | 0.2 ± 0.0 | 0.2 ± 0.1 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| 4A2 | 92.0 ± 10.8 | 147.2 ± 19.3* | 154.6 ± 14.2‡ | 150.0 ± 17.7† | 120.5 ± 4.5* | 125.1 ± 8.8 |

Values are expressed as means ± SE in pmol of P450/mg of microsomal protein (N = 6 in each group).
* $P < 0.05$, † $P < 0.02$, ‡ $P < 0.01$ and § $P < 0.001$, diabetic rats vs control rats, or insulin-treated rats vs diabetic rats without treatment.

Table 5. Changes in lauric acid hydroxylation activities of renal microsomes

| | Control | Diabetes | | | Diabetes plus insulin | |
|---|---|---|---|---|---|---|
| | | 2 week | 3 week | 5 week | 3 week | 5 week |
| Lauric acid | | | | | | |
| $\omega$ | 1.36 ± 0.12 | 2.33 ± 0.35* | 2.72 ± 0.22‡ | 2.06 ± 0.37 | 1.65 ± 0.08† | 1.53 ± 0.16 |
| $\omega - 1$ | 0.45 ± 0.04 | 0.78 ± 0.09† | 0.81 ± 0.05‡ | 0.65 ± 0.10 | 0.54 ± 0.05† | 0.48 ± 0.04 |

The catalytic activities were assayed in duplicate, and the values are expressed as means ± SE in nmol of product/min/mg of protein.

* $P < 0.05$, † $P < 0.01$ and ‡ $P < 0.001$, diabetic rats vs control rats, or insulin-treated rats vs diabetic rats without treatment.

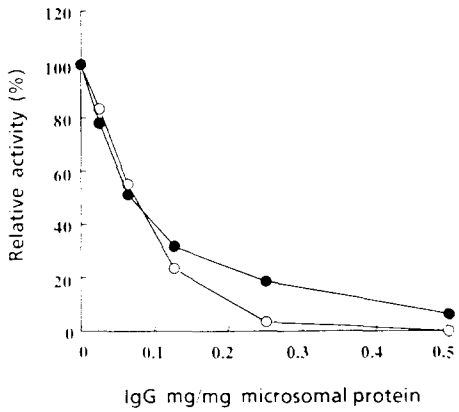


Fig. 2. Effects of anti-P450 4A2 antibody on $\omega$- (○) and ($\omega - 1$)-hydroxylation (●) activities of lauric acid in renal microsomes of diabetic rats.

diabetic rats. The differences in cytochrome P450s K-4 and 4A1 between diabetic and control rats were not significant.

Table 5 shows $\omega$- and ($\omega - 1$)-hydroxylation activities of renal microsomes in control rats and diabetic rats. Diabetic rats 2 and 3 weeks after the streptozocin injection had significantly higher $\omega$- and ($\omega - 1$)-hydroxylation activities than control rats. Diabetic rats treated with insulin for 1 week had significantly lower $\omega$- and ($\omega - 1$)-hydroxylation activities than the untreated rats with diabetes for the same time. The effects of anti-P450 4A2 antibody on $\omega$- and ($\omega - 1$)-hydroxylation activities of lauric acid in renal microsomes are shown in Fig. 2. The antibody against the renal major isozyme of cytochrome P450 4A2 completely inhibited the $\omega$-hydroxylation of lauric acid. The activity of ($\omega - 1$)-hydroxylation was also inhibited by this antibody.

## DISCUSSION

Cytochrome P450 includes a large number of isozymes that catalyse many chemical reactions and that have numerous substrates. Many isozymes catalyse multiple reactions, so the usual method of naming is inadequate for this group of heme proteins and a systematic nomenclature and classification has been devised based on structure homology of genes coding for P450 [12]. A specific cytochrome P450 isozyme, that is inducible by acetone or ethanol increases in hepatic microsmes of chemically induced or spontaneously diabetic rats [6–8, 27]. The isozyme was designated P450 2E1 by gene homology. Cytochrome P450 2E1 is induced in rats with diabetes, rats that are starved [11] and rats given a high fat diet [28], which are all ketogenic conditions, as well as in rats treated with ketones [9, 29], so elevated levels of ketone bodies seem responsible for the induction of P450 2E1. Hepatic microsomal P450 2E1 increases 2.6-fold in male rats treated by hypophysectomy, and administration of human growth hormone to hypophysectomized rats reverses the elevated hepatic P450 2E1 in these rats [30]. Diabetic rats have suppressed release of growth hormone [31] and the impaired secretion of growth hormone may also contribute to the increase in cytochrome P450 2E1 in diabetes.

Cytochrome P450 2C11 decreased in hepatic microsomes of diabetic rats. The results agree with the reduced activities of 2$\alpha$- and 16$\alpha$-hydroxylation of testosterone in diabetic rats. The elevated catalytic activity of testosterone 7$\alpha$-hydroxylation in hepatic microsomes of diabetic rats appears to be due to induction of cytochrome P450 2A1. Our results for P450 3A2 are not consistent with those reported by Thummel and Schenkman [32]; they found hepatic P450 3A2 to be suppressed in diabetic rats. This discrepancy seems to have arisen from the difference between the antibodies used. They used anti-P450 PCN-E, which recognized both P450s 3A2 and 3A1, and their data may be a composite of the changes in the two isozymes. The 2$\beta$- and 6$\beta$-hydroxylation activities of testosterone were elevated in hepatic microsomes of diabetic rats, and this change is evidence for induction of P450 3A2 in diabetes because P450 3A2 has high activities of 2$\beta$- and 6$\beta$-hydroxylation of testosterone [21].

Both P450 4A2 and 4A3 were induced in hepatic microsomes of diabetic rats, and the $\omega$- and ($\omega - 1$)-hydroxylation activities of lauric acid in hepatic microsomes were elevated. The elevation of the hydroxylation activities was almost completely inhibited by anti-P450 4A2 antibody. The induction of P450 4A2 and 4A3 seems to result from altered metabolism of fatty acids including lauric acid in

diabetes mellitus. The ω-hydroxylated fatty acids can be further oxidized into the corresponding dicarboxylic acids by alcohol dehydrogenase in microsomes. This ω-oxidation of fatty acids is usually a minor pathway of fatty acid metabolism. The physiological importance of ω-oxidation of fatty acids has not been investigated in detail, but, ω-oxidation of fatty acids is stimulated in some pathological conditions, such as diabetes and starvation [33]. Increased urinary excretion of $C_6$- and $C_8$-dicarboxylic (adipic and suberic) acids, metabolites of the ω-oxidation of medium-chain or long-chain fatty acids liberated from triglycerides has been found in diabetic rats [34] and diabetic patients with ketoacidosis [35], suggesting that the ω-oxidation of fatty acids is stimulated in diabetes. A possible function of ω-oxidation is to produce succinyl-CoA from fatty acids [36]. The monocarboxylic fatty acids liberated from triglycerides are converted to dicarboxylic acids by ω-oxidation and metabolized by β-oxidation to succinyl-CoA. In insulin-deficient conditions, acetyl-CoA from fatty acids cannot be used as a substrate in the tricarboxylic acid cycle and is changed to ketone bodies, while succinyl-CoA can be metabolized in the cycle.

The total amount of P450 in renal microsomes was lower than that in hepatic microsomes. Cytochrome P450 4A2 was the major component in renal microsomes, and cytochrome P450s 2E1, K-2, K-4, and 4A1 were detected at low concentrations. P450 2E1 was induced in renal microsomes of diabetic rats, as in hepatic microsomes. Renal P450 K-2 increased slightly but significantly in diabetic rats. This isozyme has been purified from renal microsomes of male rats, and it has hydroxylation activity toward lauric acid at the (ω − 1)-position only [24]. P450 4A2 was induced in renal microsomes of diabetic rats, and insulin treatment suppressed this induction. Elevated ω- and (ω − 1)-hydroxylation activities seem to contribute to the induction of P450 4A2, and the inhibition of these activities by anti-P450 4A2 antibody is further evidence of such a contribution. Both cytochrome P450s 4A2 and 2E1 are induced in rat renal microsomes by starvation, but treatment with acetone causes elevation of P450 2E1 only [37]. Therefore, the regulation of P450 4A2 and that of 2E1 are not the same. The biological roles of renal cytochrome P450s have not been identified, but the major renal form of P450 4A2 seems to be involved in the metabolism of fatty acids including lauric acid and arachidonic acid [24]. The induction of P450 4A2 in both renal and hepatic microsomes in diabetes indicates that the change in P450 4A2 may be systemic. Although renal cytochrome P450 isozymes are not present at high concentrations, P450s 2E1 and 4A2 in the kidney may participate in altered lipid metabolism (including ketones and fatty acids) in diabetes, as do those in the liver.

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