0026-895X/90/010119-11$02.00/0

MOLECULAR PHARMACOLOGY, 37:119–129

Effects of Testosterone and Growth Hormone Treatment on Hepatic Microsomal P450 Expression in the Diabetic Rat

KENNETH E. THUMMEL and JOHN B. SCHENKMAN
University of Connecticut Health Center, Farmington, Connecticut 06032
Received May 25, 1989; Accepted October 6, 1989

SUMMARY

The profile of hepatic microsomal cytochrome P450 expressed in the male and female rat was dramatically altered by streptozotocin-induced diabetes. In the diabetic male, P450 forms IIC11, IIC13, IIA2, and IIIA2 were suppressed and forms IIA1 and IIC12 were induced to the levels observed in the immature male rat. A 6- to 8-fold induction of P450 IIE1 was detected in both male and female diabetic rats. A member of the P450 IIIA family was also induced in the diabetic female rat. Accompanying the change in P450 profile in the diabetic male rat was a reduction in circulating testosterone and tetraiodothyronine concentrations and a sharp diminution of the normally pulsatile pattern of growth hormone secretion. In contrast to the male rat, the growth hormone secretion pattern in the diabetic female rat was unchanged from control. The hormone and P450 profiles detected in the diabetic male rat suggest a reversion to an immature physiological state. Testosterone replacement treatments carried out for 2 weeks slightly but significantly affected the suppression of P450 IIC11 and reversed the changes in P450 IIA2, IIIA2, and IIC12 in the diabetic male, without altering the suppressed state of growth hormone secretion. However, 1 week of human growth hormone, administered intravenously every 4 hr to diabetic male rats, failed to significantly reverse the diabetes-induced changes in hepatic cytochromes P450, in particular forms IIC11 and IIE1, despite the presence of an episodic plasma hGH profile. An induction of P450 IIE1 in diabetic female rats, without a reduction in growth hormone secretion, suggests that its induction in diabetes in both sexes is not related to changes in growth hormone. In addition, the results of testosterone treatment on the expression of IIC12, IIA2, and IIIA2 in the diabetic male rat suggest a regulatory role for this hormone that does not involve the pituitary secretion of growth hormone. However, the lack of effect of human growth hormone treatment in the diabetic male on levels of individual P450 forms indicates that in diabetes there may be a change in the ability of the male rat hepatocyte to respond to a somatic signal, possibly as a result of the changes in other hormone factors.

The metabolism of many xenobiotics and endogenous molecules catalyzed by the hepatic cytochrome P450 family of enzymes is altered under different physiological and pathophysiological states. In the rat, activities of some members of this enzyme system are regulated by the status of pituitary, gonadal, pancreatic, and possibly thyroid hormone secretion; these activities are manifest in striking age and sex differences in xenobiotic metabolism (1–4). Such differences in metabolism have been seen to be due to changes in levels of individual forms of cytochrome P450 in the liver microsomes (5–9). In addition to developmental changes in hepatic microsomal cytochrome P450 composition, pathophysiological conditions such as diabetes (10–12) and hypertension (13) are known to influence microsomal xenobiotic metabolism. These changes, too, appear to be exerted by alterations in hepatic microsomal cytochrome P450 composition (14–18).

The diabetes-associated changes in hepatic microsomal cytochrome P450 expression can be reversed by daily insulin replacement therapy (16–18). However, although this treatment is highly effective, it provides no indication as to the mechanism responsible for changes in the levels of the individual forms of cytochrome P450 in the diabetic rat. Favreau *et al.* (16, 18) have suggested that in the diabetic rat levels of P450IIE1 (RLM6)[1] might be modulated by metabolic ketosis associated with the disease. Levels of P450IIC11 (RLM5) have been suggested to be controlled by growth hormone (8, 9). In diabetes the pulsatile pattern of growth hormone secretion is rapidly lost, perhaps explaining the rapid disappearance of P450IIC11 and another male-specific form of P450, IIC13 (RLM3), in the diabetic male rat (17). However, other hormonal

This work was supported in part by United States Public Health Service Grant GM-26114.

[1] Individual rat liver P450 forms have been introduced by the RLM designation used in this laboratory and, when known, by their gene designation (19): P450IIA1 = RLM2b, P450IIA2 = RLM2, P-45IIC13 = RLM3, P450IIC12 = fRLM4, P450IIC11 = RLM5, P450IIE1 = RLM6, and P450IIIA2/A1 = PCN-2 (constitutive) and PCN-1 (induced) forms, respectively, recognized by anti-PCN-E.

ABBREVIATIONS: RLM6, rat liver microsomal P450 band 6 in the 45–62-KDa region of sodium dodecyl sulfate-polyacrylamide gel electrophoresis counting from anode to cathode; STZ, streptozotocin; PCN, pregnenolone-16-α-carbonitrile; hGH, human growth hormone; rGH, rat growth hormone; T_3, triiodothyronine; T_4, tetraiodothyronine; RIA, radioimmunoassay; rGRF, rat growth hormone-releasing factor.

changes such as reductions in plasma testosterone (20) and thyroid hormone (21) accompany development of diabetes, thereby complicating the picture.

In this study we have sought to evaluate the role of growth hormone and testosterone in the changes seen in individual hepatic microsomal cytochrome P450 levels in the diabetic rat and to gain a clearer understanding of the homeostatic regulation of members of this important enzyme system. Toward that goal, a STZ-treated rat model for diabetes was used, in conjunction with hormone replacement therapies.

Materials and Methods

Animal model and treatments. Male and female CD rats, 7 weeks of age, were purchased from Charles Rivers Breeding Laboratories (Wilmington, MA). The animals were housed individually in suspended metal cages and maintained on a 12 hr light/dark cycle, with standard chow and water available *ad libitum*. At 8 weeks of age, the animals were divided into control and diabetic groups. Rats in the diabetic group received a single (65 mg/kg) tail vein injection of STZ (Sigma). Rats in the control group received an equal volume of the 50 mM sodium citrate, pH 4.5, vehicle. The development of diabetes in the STZ-treated group was confirmed by positive urine glucose dip test values of 1000+ or higher (Bili Lab Stix, Ames, IN) and the lack of significant weight gain during the course of the 4-week study. Twelve days after the administration of STZ, silastic/PE-50 catheters were surgically implanted into the right jugular vein of male and female rats from both control and diabetic groups, using ethyl ether anesthesia, as previously described (22). The catheters were flushed daily with a heparin/saline solution for the duration of the study. Two weeks after the STZ dose, both catheterized and uncatheterized rats in the diabetic group were subdivided into smaller groups for hormone replacement therapy. Animals with catheters received hormone treatments for a period of 1 week. Some diabetic male and female rats remained untreated (D). Groups of diabetic male rats received one of the following treatments: 1) a single daily subcutaneous injection of 250 μg of testosterone propionate (1 mg/ml in a saline suspension) for a period of either 1 or 2 weeks (D+T); 2) 10 μg of rGRF (a bolus of 100 μg/ml in saline) (Peninsula Laboratories) via the jugular vein, every 8 hr, for a period of 1 week (D+rGRF); 3) 50 μg of hHG (2.4 units/mg) (National Hormone and Pituitary Program, National Institute of Diabetes and Diseases of the Kidney), a bolus via the jugular vein, every 4 hr, for a period of 1 week (D+hHG); or 4) 2 units of human insulin (NPH-isophane; Squibb) subcutaneously at 7 a.m. and 6 units at 5 p.m., daily, for a period of 1 or 2 weeks (D+I). One group of uncatheterized female diabetic rats received the insulin therapy described above for a period of 2 weeks.

Tissue collection. Blood was collected from uncatheterized control and diabetic male rats through the tail vein at 0, 1, 2, 3, and 4 weeks after the administration of STZ. Plasma was rapidly separated and stored at −20° for the analysis of glucose and testosterone levels. Serial blood samples were collected through the jugular vein catheter in rats from D, D+T, D+rGRF, D+hGH, and D+I treatment groups 3 weeks after the administration of STZ and 1 week after hormone therapy was initiated. Volumes of approximately 150 μl were removed every 30 min between 10 a.m. and 3 p.m. and were placed in heparinized capillary tubes. Isotonic saline was injected into the rats after each sample was taken, to replace the volume removed. Blood was immediately centrifuged and the resulting plasma was frozen on dry ice and then stored at −80° until analyzed for either rGH or hGH and, in some samples, testosterone.

All animals in this study were killed by decapitation at either 1 or 2 weeks after hormone treatments were initiated. After removal of the liver, microsomes were isolated by the method of aggregation by calcium (23) and were then stored at −80° in 0.25 M sucrose until Western blot analyses were performed. Total microsomal P450 was determined by the method of Omura and Sato (24) and protein was measured by the Biuret method (25).

Hormone analysis. Serum or plasma glucose was measured by a glucose oxidase procedure (Sigma Bulletin No. 510). Testosterone was measured in 25 or 50 μl of plasma by use of an RIA kit (Radioassay Systems Laboratories, Carson, CA) after prior triple extraction with 4:1 diethyl ether/chloroform. After extraction, the solvent was removed under N_2 and the remaining solid was redissolved in blank serum provided with the kit, at 37° for 30 min. rGH was measured in 50 μl of plasma by RIA using anti-rGH antiserum provided by Dr. A. Parlow, Pituitary Hormone Anti-sera Center and NIDDK. Growth hormone concentrations were quantitated relative to NIDDK-RP-2 standard. hGH was measured in 50 μl of plasma from D+hGH-treated rats, using a commercially available RIA kit (Quantitope HGH; Kallestad Diagnostics, Austin, TX). T_3, T_4, and T_3 uptake were measured in plasma obtained from uncatheterized male control and diabetic rats (3 weeks after STZ), also using commercially available kits (Ciba-Corning).

Western blot analysis. Seven cytochrome P450 forms were quantitated in liver microsomes by the immunochemical method of Towbin *et al.* (26), as modified by Favreau and Schenkman (18). The microsomal levels of individual P450 forms were estimated relative to previously purified standards, using polyclonal antibodies made specific by immunoabsorption or using monoclonal antibodies. Electrophoretic separation of RLM2b and RLM2 allowed quantitation of both P450 forms using anti-RLM2 IgG, as previously described (27). Monospecific anti-RLM3, -RLM5, -RLM6, and -fRLM4 have also been described (17, 18). Monoclonal anti-PCN-E (clone 2-13-1) was kindly provided by Dr. Harry Gelboin (National Institutes of Health) and has been described earlier (28). The intensity of alkaline phosphatase-mediated stained P450 bands was measured by laser densitometry.

Results

Physiological Changes in the Diabetic Rat

Circulating glucose concentrations were uniformly high in STZ-treated rats of both sexes, in comparison with controls (Table 1). Except for insulin, none of the hormone replacement treatments altered this manifestation of the diabetic state. The insulin regimen employed lowered glucose levels towards normal (male) or slightly below (female), at least at the time blood samples were taken (3 hr after the morning insulin injection).

In the STZ-treated male rat, testosterone concentrations declined steadily, compared with control, over a 4-week period (Fig. 1). Testosterone levels in diabetic rats were significantly different from control at 2, 3, and 4 weeks and by 4 weeks were approximately 34% of testosterone levels detected in age-matched controls. Within the control treatment groups, there was considerable interanimal variability (Fig. 1; Table 2). Testosterone concentrations are known to fluctuate during the course of a day (29), and this most likely accounts for the variability seen. Indeed, serial blood samples taken from control and diabetic rats (3 weeks after STZ) show an oscillation in testosterone concentration over time (Fig. 2).

The normally pulsatile pattern of growth hormone secretion in the male rat was markedly diminished following STZ injections (Fig. 3A). In some diabetic rats, blood levels reached as high as 140 ng/ml at times but, in general, growth hormone levels in the diabetic group lacked the high peaks associated with the male rat. Trough concentrations in the diabetic male, however, were still very low (Fig. 3a; Table 3), as characteristically seen in the normal adult male. Concentration-time curves of growth hormone in the adult female rat (Fig. 3B) were much flatter than those curves from the adult male. Unlike in the male rat, STZ treatment did not appear to alter the

TABLE 1

Body weight and circulating glucose concentration in diabetic and hormone-treated diabetic rats

Eight-week-old rats received either 65 mg/kg STZ (D) or citrate buffer (C). Two weeks later, groups of diabetic rats were treated with either testosterone propionate (D+T) subcutaneously, rGRF (D+rGRF) intravenously, hGH (D+hGH) intravenously, or insulin (D+I) subcutaneously, as described in Materials and Methods. Each value represents the mean of three to six rats ± standard deviation.

	Duration	C	D	D+T	D+rGRF	D+hGH	D+I
	weeks						
Male							
Body weight (g)	1	360 ± 17	251 ± 27[a]	262 ± 23[a]	236 ± 20[a]	264 ± 18[a]	294 ± 33[a]
	2	382 ± 25	251 ± 40[a]	248 ± 42[a]			
Serum glucose (mg/dl)	1	148 ± 20	491 ± 84[a]	539 ± 60[a]	559 ± 63[a]	615 ± 60[a]	188 ± 101[a]
	2	160 ± 5	494 ± 97[a]	485 ± 67[a]			
Female							
Body weight (g)	2	242 ± 7	190 ± 11[a]				233 ± 4[a,b]
Serum glucose (mg/dl)	2	162 ± 13	530 ± 31[a]				93 ± 16[a,b]

[a] Statistically different from control, $p < 0.05$ by the Student t test (Cochran's method).
[b] Statistically different from diabetic, $p < 0.05$.

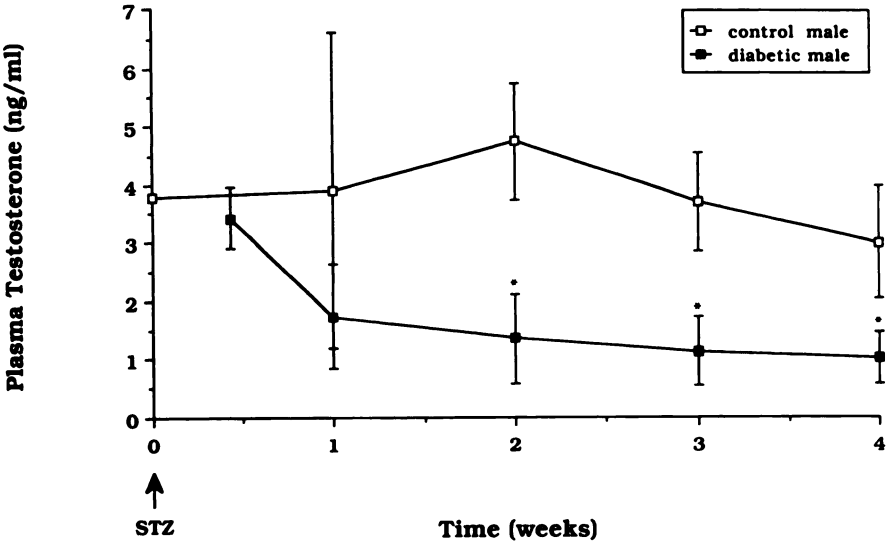

Fig. 1. Decline in serum testosterone concentration in the adult male rat, following the administration of STZ. Male 8-week-old rats were given either a single 65 mg/kg injection of STZ or sodium citrate buffer, intravenously. Blood samples were collected through a jugular vein catheter or from a tail vein, at the time intervals indicated. Testosterone was measured in plasma as described in Materials and Methods. Each *point* represents the mean ± 1 SD of 6–12 rats. *Statistically different from control, $p < 0.05$, using Student's t test (Cochran's method).

TABLE 2

Effect of hormone replacement therapy on circulating testosterone concentrations in the diabetic male rat

Treatments are described in Table 1 and in Materials and Methods. Testosterone was measured in plasma as described in Materials and Methods and its concentration is reported in ng/ml. Each value represents the mean of three to six rats ± 1 SD, except for the 1-week D+T group, which is the mean of two rats.

Duration	C	D	D+T	D+rGRF	D+hGH	D+I
weeks						
1	3.7 ± 0.8	1.1 ± 0.6[a]	2.6 ± 0.6[b]	0.6 ± 0.3[a]	0.6 ± 0.8[a]	3.4 ± 1.3[b]
2	3.0 ± 1.0	1.0 ± 0.4[a]	3.3 ± 1.0[b]			

[a] Statistically different from control, $p < 0.05$, by Student's t test (Cochran's method).
[b] Statistically different from diabetic, $p < 0.05$.

pattern of growth hormone secretion in the female rat (Fig. 3B; Table 3).

Circulating thyroxine concentrations were also measured in untreated and diabetic (3 weeks after STZ) male rats. T_4 levels dropped some 84%, from 6.1 ± 0.7 μg/dl to 1.0 ± 0.4 μg/dl, 4 weeks after the administration of STZ. No significant change in T_3 concentrations or the T_3 uptake ratio was observed, but it was noted that the results in individual rats were variable.

Hepatic P450 in the diabetic rat

Total spectrally determined cytochrome P450 in liver microsomes from diabetic male rats was significantly increased, some 42% above control (Table 4). Despite the increase in total P450, the major P450 forms normally expressed in the adult male rat, RLM5 (IIC11) and RLM3 (IIC13), were nearly absent in diabetic rats (Table 4). The microsomal content of another male-specific P450 form, RLM2 (IIA2), also declined significantly in diabetic rats, compared with control, by 3 and 4 weeks after the STZ dose. We had previously reported that the time course of decline in hepatic microsomal RLM2 in the diabetic rat proceeded at a different rate, e.g., more slowly, than the decline in RLM5 or RLM3 (30). Although there was some variability between rats, the average RLM2 levels measured in this study were 29% of control by 3 weeks after the administration of STZ (Table 4) and remained diminished by week 4. The hepatic microsomal content of P450 IIIA2 was probed using an anti-PCN-E antibody, which recognizes the constitutive form as well as the inducible form, P450 PCN-E (IIIA1). Quantitation of immunoreactive IIIA2/A1 protein is reported relative to the amount detected in untreated, age-matched, control male rats. P450 IIIA2/A1 levels measured in microsomes from untreated male rats were very variable (Table 4). This variability was

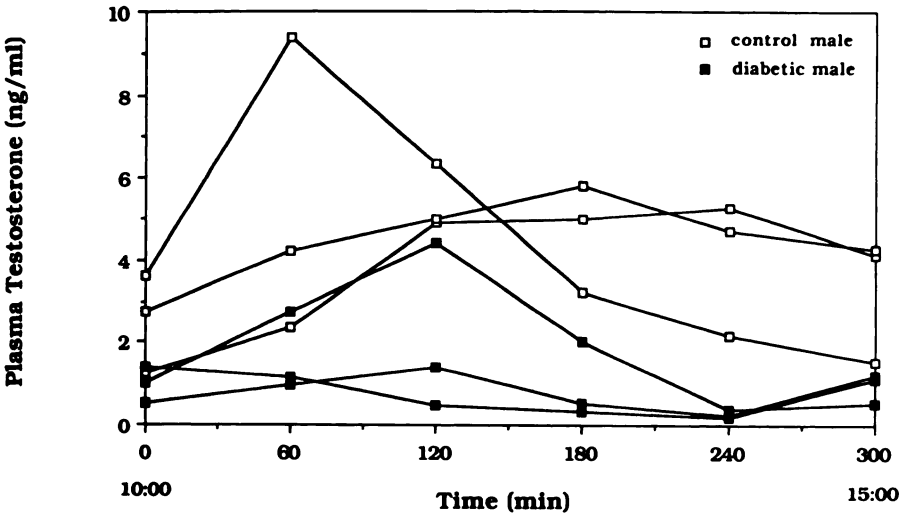

Fig. 2. Concentration-time curves of testosterone in plasma from three individual control (□) and three diabetic (■) rats. Blood samples were obtained through jugular vein catheters 3 weeks after the administration of STZ or vehicle. Testosterone was measured as described in Materials and Methods.

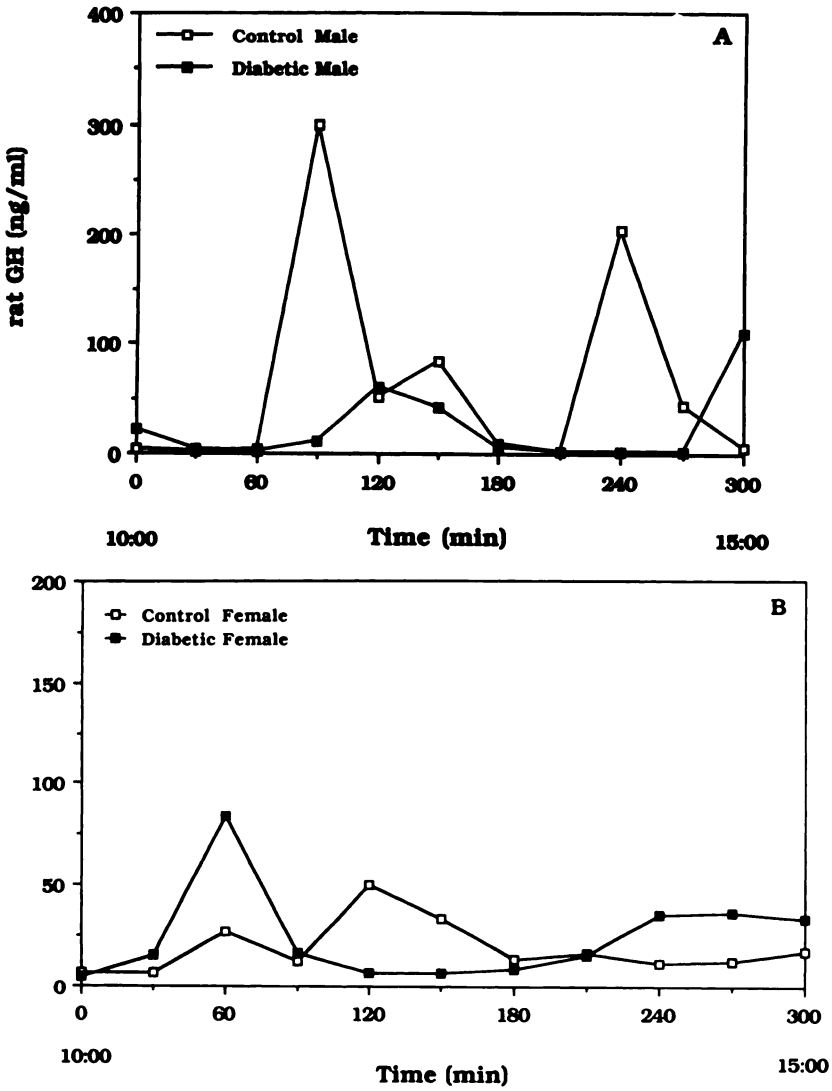

Fig. 3. Concentration-time curves of rGH in plasma from representative control (□) and diabetic (■) male (A) and female (B) rats. rGH was measured by RIA as described in Materials and Methods. Plasma samples were obtained from rats 3 weeks after the administration of STZ or vehicle. Time scale is in minutes from start of blood collection (10 a.m.), every 30 min (until 3 p.m.).

magnified in diabetic male rats; however, there was a significant decline detected in rats that were diabetic for 4 weeks, compared with controls. Because the antibody used to probe this P450 detects both the uninduced (P450 IIIA2) and induced form (P450 IIIA1), there is the possibility that the data are a composite of conflicting trends for changes in two forms of the same gene family (31).

In contrast to the male-specific P450 forms, RLM2b (IIA1) content in liver microsomes from diabetic rats increased some 3-fold by 3 weeks after STZ, compared with controls (Table 4).

TABLE 3

Effect of hormone replacement therapy on circulating growth hormone concentrations in the diabetic rat

Treatments are described in Table 1 and in Materials and Methods. rGH or hGH were measured in plasma by RIA procedures described is Materials and Methods and the concentration is reported in ng/ml. Quantitation of rGH was calculated relative to NIDDK-RP-2 standard. Values represent the mean of three to six rats ± 1 SD except for D+rGRF, which is from a single rat.

Parameter[a]	C	D	D+T	D+rGRF	D+hGH[b]	D+I
Male						
Peak concentration	272 ± 94	91 ± 66*	48 ± 3	384	2,037 ± 386*	123 ± 65*
Trough concentration	2.1 ± 0.7	1.5 ± 0.3	1.7 ± 0.5	2.1	<2.0	2.0 ± 0.3
Mean concentration	62 ± 27	18 ± 9*	14 ± 1*	30.5	172 ± 46**	26 ± 8*
Female						
Peak concentration	55 ± 51	101 ± 82				
Trough concentration	5.6 ± 1.4	6.7 ± 4.2				
Mean concentration	18 ± 9	30 ± 21				

[a] Maximum, minimum, and mean concentrations measured during a 5-hr period of blood collection between 10 a.m. and 3 p.m.
[b] hGH measured in this group.
[c] Statistically different from control, $p < 0.05$, by Student's t test (Cochran's method).
[d] Statistically different from diabetic, $p < 0.05$.

TABLE 4

Effect of supplemental hormone treatment on the hepatic microsomal content of cytochrome P450 in the STZ treated male rat

Supplemental treatments described in Table 1 were initiated 2 weeks after the administration of STZ and continued for 1 or 2 weeks as indicated. Values are mean of three to seven rats ± 1 SD.

P450 form[a]	1 Week of treatment			
	C	D	D+rGRF	D+hGH
	nmol/mg of microsomal protein			
Total P450[b]	1.01 ± 0.15	1.46 ± 0.17[c]	1.44 ± 0.07[c]	1.24 ± 0.04[c,d]
RLM2b/IIA1	0.03 ± 0.01	0.11 ± 0.03[c]	0.15 ± 0.02[c]	0.08 ± 0.02[c]
RLM2/IIA2	0.05 ± 0.01	0.01 ± 0.01[c]	0.01 ± 0.01[c]	0.03 ± 0.01[c]
RLM3/IIC13	0.26 ± 0.07	0.06 ± 0.06[c]		0.08 ± 0.05[c]
fRLM4/IIC12	ND[e]	0.04 ± 0.04	0.03 ± 0.01	ND
RLM5/IIC11	0.41 ± 0.06	0.05 ± 0.03[c]	0.02 ± 0.01	0.10 ± 0.04[c]
RLM6/IIE1	0.05 ± 0.01	0.35 ± 0.10[c]	0.30 ± 0.05[c]	0.27 ± 0.05[c]
P450IIIA2/A1	100 ± 40%	64 ± 61%		98 ± 58%
P450 Form	**2 weeks of treatment**			
	C	D	D+T	D+I
	nmol/mg of microsomal protein			
Total P450	0.92 ± 0.06	1.31 ± 0.16[c]	1.43 ± 0.03[c]	0.94 ± 0.20[d]
RLM2b/IIA1	0.03 ± 0.00	0.12 ± 0.02[c]	0.12 ± 0.01[c]	0.02 ± 0.00[d]
RLM2/IIA2	0.04 ± 0.00	0.01 ± 0.02[c]	0.04 ± 0.01[d]	0.03 ± 0.00[c]
RLM3/IIC13	0.16 ± 0.10	0.02 ± 0.02[c]	0.13 ± 0.15	0.21 ± 0.01[d]
fRLM4/IIC12	ND	0.03 ± 0.01	ND	ND
RLM5/IIC11	0.42 ± 0.09	0.02 ± 0.01[c]	0.08 ± 0.01[c,d]	0.37 ± 0.02[d]
RLM6/IIE1	0.04 ± 0.01	0.34 ± 0.06[c]	0.37 ± 0.04[c]	0.04 ± 0.01[d]
P450IIIA2/A1	100 ± 24%	41 ± 40%[c]	110 ± 17%[d]	

[a] Individual forms were quantitated by Western blot analysis, as described in Materials and Methods.
[b] Spectrally determined from the reduced P450-CO complex.
[c] Significantly different from control, $p < 0.05$, using Student's t test (Cochran's method).
[d] Significantly different from diabetic, $p < 0.05$.
[e] ND, not detected.

RLM2b is a major component of microsomal P450 in the immature male and female rat liver (approximately 14% of the P450) and, in a comparison with RLM2 (27) was found to be suppressed in the adult male following puberty. The elevation of RLM2b observed in microsomes of the diabetic male rat and the decline in the male-specific P450 forms is suggestive of a conversion to a feminine physiological state or a reversion to a more immature state. In support of this suggestion, fRLM4 (IIC12), a normally female-specific P450, was detected in microsomes from the adult diabetic male rat (Table 4) at about 3% of the P450, 3 and 4 weeks after STZ. The levels detected, however, were much lower than those in the adult female (Table 5) but are comparable to the levels found in the 4-week-old immature male rat (6, 27). A large portion of the increase in total hepatic P450 detected in microsomes from the diabetic male rat appears to be the result of a 6- to 8-fold increase in RLM6 (IIE1) content, detected at 3 and 4 weeks after STZ (Table 4). The level of RLM6 was also increased 5- to 6-fold in the diabetic female rat, in comparison with control (Table 5). RLM6 is a major P450 component of the immature male and female rat (27, 32) but declines in the maturing rat of both sexes, from a peak at 1–2 weeks to low levels when the animals are sexually mature. Peak RLM6 levels in the diabetic rat, however, were higher than peak levels observed in the 1-week-old rat.

In the female rat, diabetes did not cause any significant change in fRLM4 levels (Table 5), consistent with the above lack of change in the growth hormone profile in the diabetic female rat. RLM2b levels also did not change in the diabetic female rat, compared with control. None of the male-specific P450 forms were detected in liver microsomes from the diabetic female rat. Levels of P450 IIIA2/A1, which are very low in the

TABLE 5

Effect of supplemental hormone treatment on the hepatic microsomal content of cytochromes P450 in the STZ-treated female rat

Treatments are the same as in Table 4.

P450 form	2 weeks of treatment		
	C	D	D+I
	nmol/mg of microsomal protein		
Total P450	0.69 ± 0.09	1.20 ± 0.20[a]	0.66 ± 0.08[b]
RLM2b/IIA	0.11 ± 0.00	0.12 ± 0.02	0.07 ± 0.02[b]
RLM2/IIA2	ND[c]	ND	ND
RLM3/IIC13	ND	ND	ND
fRLM4/IIC12	0.21 ± 0.05	0.18 ± 0.05	0.19 ± 0.04
RLM5/IIC11	ND	ND	ND
RLM6/IIE1	0.06 ± 0.02	0.32 ± 0.05[a]	0.08 ± 0.01[b]
P450IIIA2/A1	7 ± 6%	47 ± 8%[a]	7 ± 5%[b]

[a] Significantly different from control, $p < 0.05$, by Student's t test (Cochran's method).
[b] Significantly different from diabetic, $p < 0.05$.
[c] ND, not detected.

adult female, relative to male, increased in the diabetic female compared with control, from 7% to 47% of normal adult male levels (Table 5), illustrating the difference in the regulation between this P450 form(s) and the other male-specific forms.

Hormone Replacement in the Diabetic Rat

In an attempt to determine the factors controlling the changing levels of the individual forms of P450, hormone replacements were implemented.

Testosterone propionate. Daily injections of testosterone propionate into the diabetic male rat increased circulating testosterone concentrations significantly, from 34% of control to 70% and 110% of control after 1 and 2 weeks of treatment, respectively (Table 2). Although daily testosterone administration restored testosterone concentrations to near normal levels, after 1 week of treatment growth hormone secretion was still suppressed in testosterone-treated diabetic (D+T) rats (Fig. 4A). There was essentially no difference in peak, trough, and mean growth hormone concentrations between diabetic and D+T rats (Table 3). Nevertheless, of the P450 forms examined, daily testosterone treatment had a statistically significant effect on RLM2, fRLM4, RLM5, and P450 IIIA2/A1 (Table 4). The microsomal contents of both RLM2 and P450 IIIA2/A1 were restored to control levels by testosterone replacement. Similarly, whereas fRLM4 appeared in the adult diabetic male rat, testosterone administration resulted in its disappearance (Table 4). Testosterone administration also partially restored RLM5 content in diabetic rat microsomes (Table 4) to a level 4-fold higher than the content in microsomes from diabetic male rats.

rGRF. The reduction in growth hormone secretion from the pituitary of STZ-treated male rats is thought to be the result of constant pituitary suppression by high circulating concentrations of somatostatin, which arise from extrahypothalamic sources (33). rGRF was administered intravenously to diabetic male rats in an effort to override the effects of somatostatin and cause a normal release of growth hormone from the pituitary. Because of a limited supply of releasing factor, rGRF was administered every 8 hr for 1 week. rGRF did cause a pulsed release of growth hormone (Fig. 4B) but, although the magnitude of the peak was quite high, the duration was brief. Growth hormone concentrations rapidly dropped to trough concentrations within 90 min after the rGRF dose was administered. In

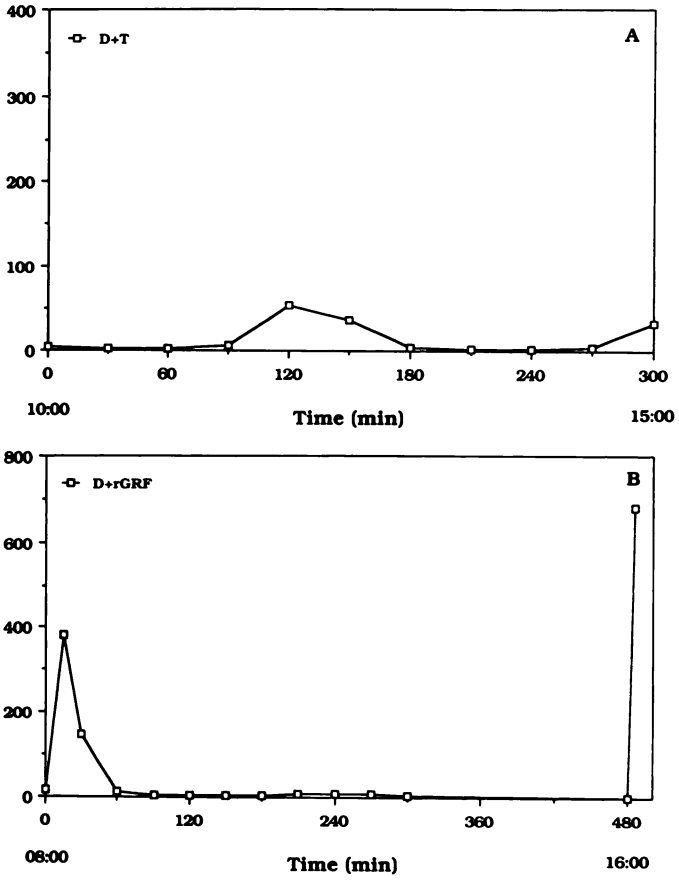

Fig. 4. Concentration-time curves of growth hormone in plasma obtained from individual male diabetic rats that received testosterone (A) or rGRF (B) therapy for a period of 1 week, as described in Materials and Methods. Serial blood samples were collected 3 weeks after the administration of STZ (at the end of the 1-week treatment period). rGH was quantitated by RIA as described in Materials and Methods. The plasma testosterone concentration in the rat represented in A was 3.1 ng/ml at the time the last serial blood sample was collected. rGRF was administered to the rat represented in B at 8 a.m. and at 4 p.m.

addition, over the next 6.5 h an even greater degree of growth hormone suppression than was seen in the diabetic male occurred (contrast Fig. 4B and Fig. 3A). Not unexpectedly, circulating testosterone concentrations in D+rGRF rats were quite low, 16% of control levels, and comparable to the levels seen in matched diabetic rats (Table 2). Despite the episodic pattern of growth hormone concentration in diabetic rats given the releasing factor, 1 week of treatment did not change the profile of hepatic microsomal P450 in D+rGRF from that found in diabetic male rats (Table 4).

hGH. In an effort to circumvent the pituitary and still simulate a male pattern of growth hormone concentration, hGH was administered intravenously to diabetic rats, every 4 hr, for a period of 1 week. Peak hGH concentrations following the 50-μg dose were 7.5-fold higher than the peak rGH levels detected in normal adult male rats (Fig. 5A; Table 3). Over the next 2 to 3 hr, peak hGH concentrations rapidly decreased to below 4 ng/ml. In general, the periods of high and low growth hormone concentrations seemed comparable to those found in the normal adult male rat (Fig. 3A). Treatment with hGH did not cause an increase in circulating testosterone concentration above that observed in diabetic rats (Table 2).

Although a male pattern of growth hormone concentrations

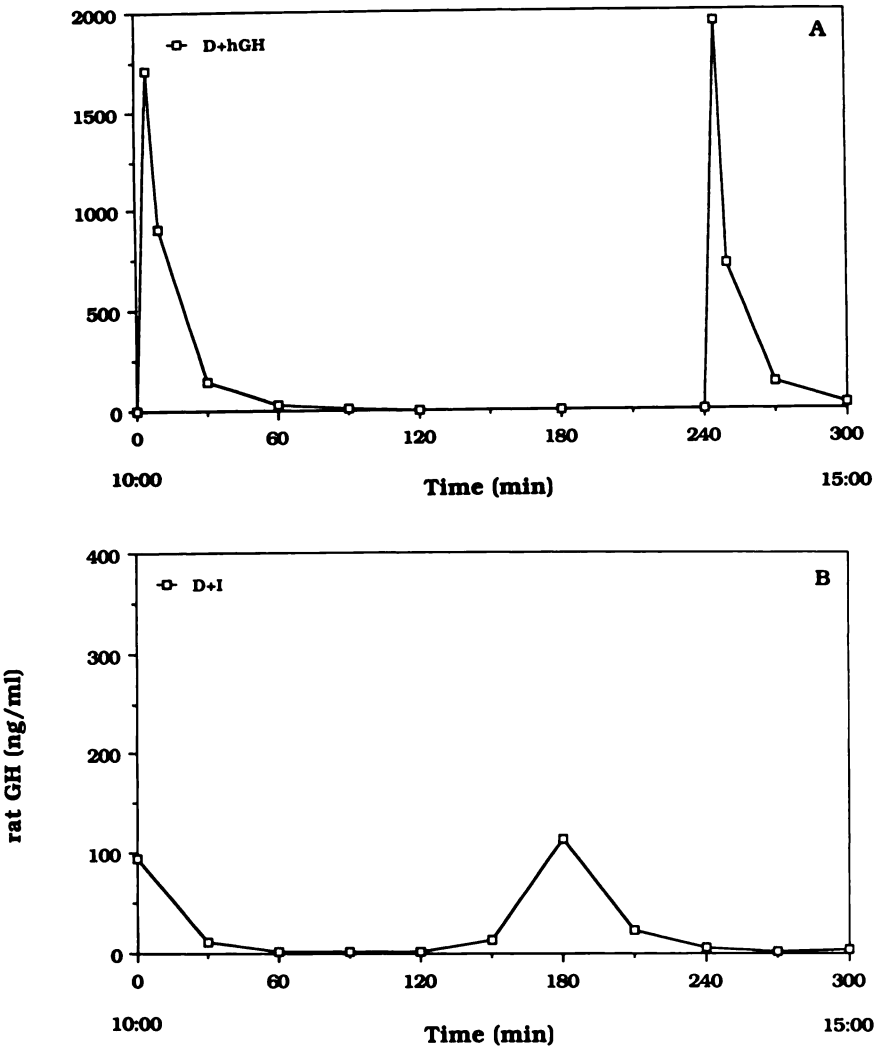

Fig. 5. Concentration-time curves of hGH (A) or rGH (B) in plasma obtained from individual diabetic rats that received either hGH or insulin therapy, respectively. Hormone treatments and measurements are described in Materials and Methods. Blood samples were collected 3 weeks after the administration of STZ (at the end of the 1-week treatment period). hGH was administered to the rat represented in A at 10 a.m. and 2 p.m. Insulin (2 units) was administered to the rat represented in B at 7 a.m.

was achieved in D+hGH rats, the profile of cytochrome P450 forms measured in liver microsomes was essentially no different from the diabetic male profile (Table 4). Individually, the levels of RLM2b, RLM2, RLM5, RLM6, and P450 IIIA2/A1 all changed from the diabetic profile towards the profile of a normal male rat, but none of these changes proved statistically significant. P450 form fRLM4 was undetected in liver microsomes from hGH-treated diabetic rats, an effect also observed following testosterone treatments.

Insulin. Insulin treatment represented a positive control in this study. Daily injections for 1 week restored circulating testosterone concentrations of the D+I male rat to normal (Table 2). The effect of insulin on growth hormone secretion, however, was less than expected in this period (Fig. 5B). Peak and mean growth hormone concentrations in D+I rats were only marginally higher than the levels in diabetic rats (Table 3). Blood samples collected in this study were drawn between 3 and 8 hr after the lower morning insulin dose. In one animal, blood samples were drawn in the evening after the larger of the two daily insulin injections had been administered and while the animal was actively feeding. Again, both the peak and mean growth hormone concentrations were 1/2 the average peak and mean concentrations in the control male group. Nevertheless, the insulin-treated diabetic male rat had a hepatic P450 profile that was essentially the same as that of the control male rat (Table 4). In particular, the levels of the most responsive forms, RLM5 and RLM6, in microsomes from D+I rats were 88% and 100% of control, respectively. Insulin treatment for 2 weeks also reversed the effect of diabetes on microsomal RLM6 and P450 IIIA2/A1 content in the female rat (Table 5).

Discussion

Rat liver microsomal cytochrome P450 is a family of adaptive enzymes, several of which responded to the pathophysiological changes that occur in diabetes. The individual forms of P450 responded differently to the diabetic conditions in both male and female rats.

The overall pattern of changes in hepatic microsomal P450 expression in the diabetic male rat is suggestive of a reversion to an immature physiological state where high growth hormone pulses are absent and testosterone concentrations are lower than in the adult. Although there are many similarities in the P450 profile expressed in the diabetic male and the adult female rat, both the low level of IIC12 and the low trough growth hormone concentrations detected in the diabetic male rat do not necessarily indicate a feminization of the rat. All of the changes in hepatic microsomal P450 detected in the diabetic male rat (Table 4) (18, 34, 35) and in the diabetic female rat

(Table 5) are reversed by daily injections of insulin. Although insulin alone is effective, the possibility that its actions are indirect was addressed using other hormone replacements.

Of the P450 forms examined in this study, only IIE1 levels were altered by diabetes to a similar degree and direction in both male and female rats. This finding is significant, given the distinctly different patterns of growth hormone secretion (Fig. 3) detected in the diabetic male (low troughs and low peaks) and normal and diabetic female (high troughs and low peaks). If P450 IIE1 expression in the adult rat is inhibited by growth hormone (36), then its elevation in diabetes would be expected only in the male rat. A sex difference was not observed, and it seems more likely that the elevation of IIE1 is a response to metabolic ketosis in both male and female rats. To some extent, the diabetic rat resembles the 2-week-old rat in that microsomal IIE1 levels are high (compared with the newborn and adult) and fat serves as a major energy source. A state of metabolic ketosis exists in both the nursing pups (37) and adult diabetic rats (12, 38). In addition, the rapid rise and subsequent decline in IIE1 levels in the immature rat, between the ages of 0 and 4 weeks (30, 32), correspond to the active nursing and then weaning phases in the rat's life, respectively, and are also accompanied by a concomitant rise and then fall in circulating ketone body concentrations (37).

A mechanistic role for growth hormone in the induction of IIE1 in the diabetic male rat has not been completely ruled out. The lack of a significant effect of growth hormone replacement treatments on any of the diabetes-induced changes in hepatic P450, particularly IIC11, suggests that in our study the growth hormone signal was not received or was not interpreted properly by the diabetic rat hepatocytes. More elaborate hormone treatments may be needed to demonstrate suppression of (or lack of) IIE1 by growth hormone in the diabetic rat.

There is considerable evidence in the literature indicating a regulatory role for growth hormone in the expression of P450 IIC11 in the male rat (8, 9, 39). The decline in this enzyme content in the diabetic male rat is most likely a result of a change in the pattern of growth hormone secretion from the pituitary, which results in a reduced peak and mean plasma level (Fig. 3A). Two growth hormone replacement treatments were employed in order to confirm this hypothesis. Neither treatment was effective in restoring IIC11 levels. Although it can be argued that the periodicity of growth hormone peaks in diabetic male rats receiving rGRF was too great for effective IIC11 expression (Fig. 4B), it was surprising to find hGH treatment equally ineffective at restoring the levels, given the hGH concentration-time curves obtained in the latter animals (Fig. 5A). Hypophysectomized male rats treated with hGH on an even less rigorous intermittent schedule (every 12 hr) reportedly expressed IIC11 at near normal levels (8, 39). There are similarities between the hypophysectomized and diabetic animal models, but significant differences exist between them. The former rat does not secrete growth hormone, and it similarly lacks all of the pituitary trophic hormones, e.g., follicle-stimulating hormone and luteinizing hormone, both of which are diminished but present in the diabetic rat (20). Thyroid function is also diminished in both diabetic (21) and hypophysectomized rats. However, very low concentrations of insulin and probably high concentrations of somatostatin are present in the STZ-induced diabetic male rat (33, 40). Indeed, it is the secretion of somatostatin from nonhypothalamic tissues that is thought to cause the substantial inhibition of growth hormone release from the pituitary in a diabetic male rat (33). The lack of a growth hormone effect on P450IIC11 levels in D+hGH-treated rats may conceivably be due to inhibition by the supraphysiological levels achieved or to a decline in hepatic somatogenic receptor numbers or their inability to transmit a growth hormone signal, the latter as a result of low insulin and/or high somatostatin concentrations. A decrease in somatogenic receptor numbers in diabetic male and female rats has been reported (41) and insulin treatment also reverses this change. The fact that diabetic male rats treated with insulin showed nearly normal levels of IIC11 and yet only modest peak growth hormone concentrations further suggests that increased insulin concentrations and/or reductions in somatostatin concentration were important factors in the reexpression of IIC11 to normal adult levels and that growth hormone alone is not sufficient for its regulation.

Another indicator of a restored "male" physiology in the insulin-treated diabetic rat was the measurement of a normal adult male testosterone concentration. Whether this was also mechanistically important for the elevation in hepatic IIC11 content in D+I rats is not clear. Although testosterone is an important regulator of normal growth hormone secretion in the adult male rat (42), growth hormone secretion in the D+I male rat was still reduced compared with control (Fig. 5B), despite the normal adult testosterone levels. Further, although testosterone concentrations in D+T rats similar to those in control male rats did result in a small but statistically significant increase in IIC11 levels, compared with levels in diabetic rats, the suppressed state of growth hormone secretion was not altered. Interestingly, in the neonatally castrated adult male rat, the IIC11 level was found to be very low, and testosterone administered for 1 week restored it to normal levels (43).

A stronger case can be made for a testosterone effect on hepatic IIC12 content in the diabetic rat. As mentioned above, the levels of IIC12 in the diabetic male rat at weeks 3 and 4 after STZ were similar to the levels found in the immature male rat. The diabetic male rat lacked the continuous growth hormone secretion necessary for a higher level of expression of IIC12 such as that found in the adult female rat (7) but did show low intermittent bursts of growth hormone secretion. It is possible that in both cases, immature and diabetic rats, growth hormone secretion in transition to or from an adult pattern triggers a limited expression of IIC12. However, the time course of change in growth hormone secretion after the administration of STZ is reportedly rapid (33), in contrast to the 2–3-week delay for the appearance of IIC12 (30). The time course of decline in testosterone concentration (Fig. 1), however, followed more closely the appearance of IIC12 (Fig. 1). In the adult male rat, testosterone is thought to be important in maintaining low trough growth hormone concentrations through its effects on hypothalamic control of pituitary growth hormone secretion (42). A prolonged absence of testosterone following prepubertal gonadectomy will reportedly result in IIC12 (P450-female) expression in the adult male rat (43). In the current study, treatment of diabetic male rats daily with testosterone propionate achieved physiological testosterone concentrations and completely prevented the appearance of IIC12 (Table 4). However, this treatment did not affect the pattern of growth hormone secretion (Fig. 3A versus Fig. 4A). Therefore, the effect of testosterone on IIC12 expression would

seem to be more direct rather than through the hypothalamo-pituitary. It is suggested that the basal level of IIC12 expressed in the diabetic rat is similar to the level found in the immature rat and that it is inhibited by testosterone (found in the adult and D+T male) or is induced by a pattern of continuous growth hormone secretion such as is found in the adult female rat.

P450IIA2 expression in the diabetic male rat was also altered by testosterone treatment. Daily injections of testosterone propionate restored it to control male levels, suggesting that the androgen either directly or indirectly stimulates IIA2 synthesis or slows its rate of degradation. This enzyme is also a neonatally imprinted, male-specific, P450 form detected in the developing rat only after the onset of puberty (27, 44). Hepatic levels of IIA2 in the adult male rat, however, were much lower than the levels of other male-specific P450 forms, i.e., IIC11 or IIC13. In addition, Waxman *et al.* (44) have reported that IIA2 was not regulated by an episodic pattern of growth hormone secretion, but rather was inhibited by growth hormone both in males and females, and that only the low trough concentrations in the male allowed some IIA2 to be expressed. Hypophysectomized male and female rats reportedly had higher IIA2 levels than their respective controls and, at least in males, intermittent infusion with growth hormone partially reversed the increase (44). In our studies, a decline in hepatic IIA2 was observed by 3 and 4 weeks after the administration of STZ in the diabetic male (Table 4), despite the markedly depressed growth hormone concentrations. The data suggest the possibility of two conflicting effects, i.e., the disappearance of an inhibitor, growth hormone, and the loss of a normally stimulatory or maintenance factor, testosterone. IIA2 was not detected in microsomes from the diabetic female rat, consistent with the lack of change in growth hormone concentrations observed and the reported inhibitory properties of this hormone.

The regulation of P450 IIIA2, another constitutive male-specific P450 form, is reported to be remarkably similar to the regulation of IIA2, and growth hormone has been shown to have an inhibitory effect on its expression as well (44, 45). P450 IIIA2 is 89% homologous (31) to a PCN-inducible form, IIIA1 (PCN2, P450 PB-2a). The antibody used as a probe in the current study, anti-PCN-E (clone 2-13-1, courtesy of Dr. H. V. Gelboin), recognizes both forms, but only IIIA2 is present in the untreated adult male (31, 44). In general, the effect of diabetes on the content of IIIA forms in the male rat is similar to its effect on IIA2. A considerable amount of variability between animals in the diabetic groups was encountered, making the results almost uninterpretable. However, 4 weeks after the administration of STZ, P450 IIIA2/A1 levels detected in the diabetic male were significantly lower than the levels in age-matched controls ($p < 0.05$), in contrast to the elevation of IIIA2 seen after hypophysectomy (44, 45). The apparent discrepancy was resolved by the observation that testosterone treatment prevented this decline (Table 4). This, as was the case with IIA2, indicates a possible direct or indirect role for androgen in regulating levels of the constitutive form IIIA2. Variability in the amount of P450 IIIA2/A1 protein detected in the diabetic group may reflect conflicting regulatory signals. The absence of IIIA2 inhibition by growth hormone would result in its elevation, and the reduction in levels of testosterone would result in its suppression. However, it is not known whether IIIA1 or another closely related member of the same gene family also responds to the effects of diabetes. The possibility of an increase in levels of P450 IIIA1 or another closely related form is suggested by the substantial (7-fold) increase in immunoreactive protein in hepatic microsomes from the diabetic female rat, in which the appearance of IIIA2 was not expected because the continuous female pattern of growth hormone secretion was maintained despite the diabetic state.

A P450 protein that reacts with anti-P450 IIIA1 antibody is found in hepatic microsomes from 1–2-week-old rats of both sexes (31, 44). It has been suggested (44) that this form, which declines in both sexes before puberty, may be distinctly different from P450 IIIA2, which appears in the male rat after puberty much as IIA2 does, and is different from P450 IIIA1, whose mRNA is not detected in normal male or female rats of any ages (31). The protein detected in the diabetic female rat, and possibly a portion of the protein detected in the diabetic male by anti-PCN-E, may represent the same P450 form found in the 2-week-old rat.

Although it is not a sex-specific P450 form in the rat, P450 IIA1 does exhibit differential expression in the adult, with much lower levels detected in liver microsomes of the male rat than the female rat. The decline in IIA1 in the developing male rat occurs just before and during puberty (5, 27, 46) and might be the result of inhibition by developing high peak concentrations of growth hormone or of decreased synthesis during periods of low trough growth hormone concentrations. The observed rise in IIA1, detected as early as 3 days after STZ (data not shown) in the diabetic male but not in the diabetic female, is consistent with an inhibition by growth hormone.[2] Restoration of physiological testosterone concentrations without changing growth hormone secretion did not alter the degree of IIA1 elevation in diabetes and indicates that testosterone is not responsible for the lower level of IIA1 in the pubescent or sexually mature male rat.

In a paper that has just appeared, Yamazoe *et al.* (47) reported on the administration of growth hormone, somatomedin C, and insulin to the diabetic male rat and their effects on P450-male (P450 IIC11), P450 6β-1 (P450 IIIA2) P450b and e (P450 IIB1 and IIB2), and P448H expression in liver microsomes. These investigators found that twice-daily injections of growth hormone to the diabetic male rat had no effect on the suppressed levels of P450-male, in agreement with the above results, whereas the same treatment given to the hypophysectomized male rat restored hepatic P450-male content towards the level of a normal control. P450 IIIA2 was also found to decline during the early weeks of diabetes but rebounded back to control levels 5 weeks after alloxan treatment.

In conclusion, diabetes is a pathophysiological state that is useful as a probe of microsomal hepatic cytochrome P450 regulation in the rat. Except for P450 IIE1, all of the changes we report in P450 expression in the diabetic rat appear to arise from a disruption of the sexually dimorphic physiological states that are imprinted just after birth and develop during puberty. The suppression of IIC11, IIC13, IIA2, and IIIA1/A2 and the induction of IIA1 and IIC12, all in the diabetic male rat, correlate well with changes in the secretion of either growth hormone, testosterone, or some related factor that is altered during puberty. In contrast, the induction of IIE1 in the diabetic

[2] While this paper was undergoing review a paper has appeared [D. J. Waxman, J. J. Morrissey, and G. J. LeBlanc, *Endocrinology* **124**:2954 (1989)] that suggests that high growth hormone pulses may suppress levels of IIA1.

rat is again suggested to be the result of a state of metabolic ketosis associated with the disease, as previously reported (16, 18, 35). The inability of the hormone replacement treatments employed to fully reverse most of the effects on cytochrome P450 forms limits the conclusions but also points to the complexity associated with the regulation of this hepatic enzyme system. Although most of the forms of P450 used reflect sex-specific forms without human orthologs, thereby limiting extrapolation of results reported from studies of the diabetic rat to humans, some forms such as IIE1 do have orthologs in human and other species, making studies such as provided in this paper useful in conceptualizing roles for the individual forms.

Acknowledgments

The authors wish to thank Ms. Wendy VanSickle for her excellent technical assistance, Dr. Sid Hopfer and Dr. Eva Horak for their sound advice and helpful discussions, and Dr. Ingela Jansson for her critical review of this manuscript.

References

1. Conney, A. H., K. Schneidman, M. Jacobson, and R. Kuntzman. Drug-induced changes in steroid metabolism. *Ann. N. Y. Acad. Sci.* **123**:98–109 (1965).
2. Schenkman, J. B., I. Frey, H. Remmer, and R. W. Estabrook. Sex differences in drug metabolism by rat liver microsomes. *Mol. Pharmacol.* **3**:516–525 (1967).
3. Kato, R. Sex-related differences in drug metabolism. *Drug Metab. Rev.* **3**:1–32 (1974).
4. Gustafsson, J.-A., and A. Stenberg. On the obligatory role of the hypophysis in sexual differentiation of hepatic metabolism in rats. *Proc. Natl. Acad. Sci. USA* **73**:1462–1465 (1976).
5. Thomas, P. E., L. M. Reik, D. E. Ryan, and W. Levin. Regulation of three forms of cytochrome P450 and epoxide hydrolase in rat liver microsomes. *J. Biol. Chem.* **256**:1044–1052 (1981).
6. Maeda, K., T. Kamataki, T. Nagai, and R. Kato. Postnatal development of constitutive forms of cytochrome P450 in liver microsomes of male and female rats. *Biochem. Pharmacol.* **33**:509–512 (1984).
7. MacGeoch, C., E. T. Morgan, and J.-A. Gustafsson. Hypothalamo-pituitary regulation of cytochrome $P450_{16\beta}$ apoprotein levels in rat liver. *Endocrinology* **117**:2085–2092 (1985).
8. Morgan, E. T., C. MacGeoch, and J.-A. Gustafsson. Hormonal and developmental regulation of expression of the hepatic microsomal steroid 16α-hydroxylase cytochrome P450 apoprotein in the rat. *J. Biol. Chem.* **260**:11895–11898 (1985).
9. Kamataki, T., M. Shimada, K. Maeda, and R. Kato. Pituitary regulation of sex-specific forms of cytochrome P450 in liver microsomes of rats. *Biochem. Biophys. Res. Commun.* **130**:1247–1253 (1985).
10. Dixon, R. L., L. G. Hart, and J. R. Fouts. The metabolism of drugs by liver microsomes from alloxan-diabetic rats. *J. Pharmacol. Exp. Ther.* **133**:7–11 (1961).
11. Kato, R. Drug metabolism under pathological and abnormal physiological states in animals and man. *Xenobiotica* **7**:25–92 (1977).
12. Miller, K. W., and C. S. Yang. Studies on the mechanisms of induction of *N*-nitrosodimethylamine demethylase by fasting, acetone, and ethanol. *Arch. Biochem. Biophys.* **229**:483–491 (1984).
13. Greenspan, P., and J. Baron. Hepatic microsomal oxidative drug metabolism in the spontaneously hypertensive rat. *Biochem. Pharmacol.* **30**:678–681 (1981).
14. Past, M. R., and D. E. Cook. Effect of diabetes on rat liver cytochrome P450: evidence for a unique diabetes-dependent rat liver cytochrome P450. *Biochem. Pharmacol.* **31**:3329–3334 (1982).
15. Rouer, E., P. Beaune, and J. P. Leroux. Immunoquantitation of some cytochrome P450 isozymes in liver microsomes from streptozotocin-diabetic rats. *Experientia (Basel)* **42**:1162–1163 (1986).
16. Favreau, L. V., D. M. Malchoff, J. E. Mole, and J. B. Schenkman. Responses to insulin by two forms of rat hepatic microsomal cytochrome P450 that undergo major (RLM6) and minor (RLM5b) elevations in diabetes. *J. Biol. Chem.* **262**:14319–14326 (1987).
17. Favreau, L. V., and J. B. Schenkman. Decrease in the levels of a constitutive cytochrome P450 (RLM5) in hepatic microsomes of diabetic rats. *Biochem. Biophys. Res. Commun.* **142**:623–630 (1987).
18. Favreau, L. V., and J. B. Schenkman. Compositional changes in hepatic microsomal cytochrome P450 during onset of streptozocin-induced diabetes and during insulin treatment. *Diabetes* **37**:577–584 (1988).
19. Nebert, D. W., J. R. Nelson, M. Adesnik, M. J. Coon, R. W. Estabrook, F. J. Gonzalez, F. P. Guengerich, I. C. Gunsalus, E. F. Johnson, B. Kemper, W. Levin, I. R. Phillips, R. Sato, and M. R. Waterman. The P450 gene superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA* **8**:1–13 (1989)
20. Murray, F. T., J. Orth, G. Gunsalus, J. Weisz, J. B. Li, L. S. Jefferson, N. A. Musto, and C. W. Bardin. The pituitary-testicular axis in the streptozotocin diabetic male rat: evidence for gonadotroph, Sertoli cell and Leydig cell dysfunction. *Int. J. Androl.* **4**:265–280 (1981).
21. Chopra, I. J., W. Wiersinga, and H. Frank. Alterations in hepatic monoiodination of iodothyronines in the diabetic rat. *Life Sci.* **28**:1765–1776 (1981).
22. Thummel, K. E., J. T. Slattery, and S. D. Nelson. Mechanism by which ethanol diminishes the hepatotoxicity of acetaminophen. *J. Pharmacol. Exp. Ther.* **245**:129–136 (1988).
23. Schenkman, J. B., and D. L. Cinti. Preparation of microsomes with calcium. *Methods Enzymol.* **52**:83–89 (1978).
24. Omura, T., and R. Sato. A new cytochrome in liver microsomes. *J. Biol. Chem.* **237**:PC1375–PC1376 (1962).
25. Gornall, A. G., C. J. Bardawill, and M. M. David. Determination of serum proteins by means of the Biuret reaction. *J. Biol. Chem.* **177**:751–766 (1949).
26. Towbin, H., T. Staehelin, and J. Gordon. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**:4350–4354 (1979).
27. Thummel, K. E., L. V. Favreau, J. E. Mole, and J. B. Schenkman. Further characterization of RLM2 and comparison with a related form of cytochrome P450, RLM2b. *Arch. Biochem. Biophys.* **266**:319–333 (1988).
28. Park, S. S., D. J. Waxman, H. Miller, R. Robinson, C. Attisano, F. P. Guengerich, and H. Gelboin. Preparation and characterization of monoclonal antibodies to pregnenolone 16-α-carbonitrile inducible rat liver cytochrome P-450. *Biochem. Pharmacol.* **35**:2859–2867 (1986).
29. Ellis, G. B., and C. Desjardins. Male rats secrete luteinizing hormone and testosterone episodically. *Endocrinology* **110**:1618–1627 (1982).
30. Schenkman, J. B., K. E. Thummel, and L. V. Favreau. Homeostatic changes in rat hepatic microsomal cytochrome P450, in *Cytochrome P450: Biochemistry and Biophysics* (I. Schuster, ed.). Taylor and Francis, New York, 851–854 (1989).
31. Gonzalez, F. J., B.-J. Song, and J. P. Hardwick. Pregnenolone 16α-carbonitrile-inducible P450 gene family: gene conversion and differential regulation. *Mol. Cell. Biol.* **6**:2969–2976 (1986).
32. Thomas, P. E., S. Bandiera, S. L. Maines, D. E. Ryan, and W. Levin. Regulation of cytochrome P450j, a high-affinity *N*-nitrosodimethylamine demethylase, in rat hepatic microsomes. *Biochemistry* **26**:2280–2289 (1987).
33. Tannenbaum, G. S. Growth hormone secretory dynamics in streptozotocin diabetes: evidence of a role for endogenous circulating somatostatin. *Endocrinology* **108**:76–82 (1981).
34. Dong, Z., J. Hong, Q. Ma, D. Li, J. Bullock, F. J. Gonzalez, S. S. Park, H. V. Gelboin, and C. S. Yang. Mechanism of induction of cytochrome P450ac (P450j) in chemically induced and spontaneously diabetic rats. *Arch. Biochem. Biophys.* **263**:29–35 (1988).
35. Bellward, G. D., T. Chang, B. Rodrigues, J. H. McNeill, S. Maines, D. E. Ryan, W. Levin, and P. E. Thomas. Hepatic cytochrome P450j induction in the spontaneously diabetic BB rat. *Mol. Pharmacol.* **33**:140–143 (1988).
36. Williams, M. T., and L. C. Simonet. Effects of growth hormone on cytochrome P450j. *Biochem. Biophys. Res. Commun.* **155**:392–397 (1988).
37. Lockwood, E. A., and E. Bailey. The course of ketosis and the activity of key enzymes of ketogenesis and ketone-body utilization during development of the postnatal rat. *Biochem. J.* **124**:249–254 (1971).
38. Schenkman, J. B., K. E. Thummel, and L. V. Favreau. Physiological and pathophysiological alterations in rat hepatic cytochrome P-450, *Drug Metab. Rev.* **20**:557–584 (1989).
39. Yamazoe, Y., M. Shimada, T. Kamataki, and R. Kato. Effects of hypophysectomy and growth hormone treatment on sex-specific forms of cytochrome P450 in relation to drug and steroid metabolisms in rat liver microsomes. *Jpn. J. Pharmacol.* **42**:371–382 (1986).
40. Patel, Y. C., T. Wheatley, F. Malaisee-Lagae, and L. Orci. Elevated portal and peripheral blood concentration of immunoreactive somatostatin in spontaneously diabetic (BBL) Wistar rats: suppression with insulin. *Diabetes* **29**:757–761 (1980).
41. Baxter, R. C., J. M. Bryson, and J. R. Turtle. Somatogenic receptors of rat liver: regulation by insulin. *Endocrinology* **107**:1176–1181 (1980).
42. Jansson, J.-O., S. Eden, and O. Isaksson. Sexual dimorphism in the control of growth hormone secretion. *Endocrine Rev.* **6**:128–150 (1985).
43. Shimada, M., N. Murayama, Y. Yamazoe, T. Kamataki, and R. Kato. Further

studies on the persistence of neonatal androgen imprinting on sex-specific cytochrome P450, testosterone and drug oxidations. *Jpn. J. Pharmacol.* **45**:467–478 (1987).

44. Waxman, D. J., G. A. LeBlank, J. J. Morrissey, J. Staunton, and D. P. Lapenson. Adult male-specific and neonatally programmed rat hepatic P450 forms RLM2 and 2a are not dependent on pulsatile plasma growth hormone for expression. *J. Biol. Chem.* **263**:11396–11406 (1988).
45. Yamazoe, Y., M. Shimada, N. Murayama, S. Kawano, and R. Kato. The regulation by growth hormone of microsomal testosterone 6β-hydroxylase in male rat livers. *J. Biochem.* **100**:1095–1097 (1986).
46. Nagata, K., T. Matsunaga, J. Gillette, H. V. Gelboin, and F. J. Gonzalez. Rat testosterone 7α-hydroxylase: isolation, sequence, and expression of cDNA and its developmental regulation and induction by 3-methylcholanthrene. *J. Biol. Chem.* **262**:2787–2793 (1987).
47. Yamazoe, Y., N. Murayama, M. Shimada, K. Yamauchi, and R. Kato. Cytochrome P450 in livers of diabetic rats: regulation by growth hormone and insulin. *Arch. Biochem. Biophys.* **268**:567–575 (1989).

Send reprint requests to: John B. Schenkman, University of Connecticut Health Center, Farmington, CT 06032.