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Suppression of Hepatic Levels of an Ethanol-Inducible P-450DM/j by Growth Hormone: Relationship between the Increased Level of P-450DM/j and Depletion of Growth Hormone in Diabetes

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SUMMARY

The mechanism of the suppression of an ethanol-inducible cytochrome P-450 (P-450DM/j) by pituitary hormone has been studied in rats. The hepatic content of P-450DM/j protein quantitated by Western blots was low but was 2-fold higher in male than female untreated rats (75 and 34 pmol/mg of protein, respectively). The content was increased 2.6-fold (male) and 5.6-fold (female) by hypophysectomy and the sex-related difference was abolished. Treatment of hypophysectomized rats with human growth hormone (hGH), but not with prolactin, reversed the increased amounts of P-450DM/j protein. The hGH-induced suppression was more effective with the continuous infusion than intermittent injection. The hepatic level of P-450DM/j mRNA, determined by the use of a 23-mer oligonucleotide probe, was also changed by hypophysectomy and/or hGH-treatment, largely in parallel with the changes in the content of P-450DM/j protein and microsomal *p*-nitrophenol and aniline hydroxylations. These results suggest that growth hormone exerts the suppressive effect on P-450DM/j through a somatogenic receptor-mediated process. In another growth hormone-depleted condition, diabetes, the hepatic level of P-450DM/j mRNA was also increased to a level similar to that in hypophysectomized rats, but the protein content was 2- to 3-fold higher in diabetic than hypophysectomized rats. These results indicate, in addition to the reduction of serum growth hormone level, the presence of another stimulatory factor, which acts translationally or posttranslationally in livers of diabetic rats. On the other hand, coordinate changes in the level of P-450DM/j protein and the mRNA in hypophysectomized rats indicate that growth hormone acts rather directly and suppresses the level of P-450DM/j mainly at a pretranslational step in rat livers.

Recent studies on the expression and regulation of hepatic P-450[1] indicate that the change in hormonal levels is a major factor in the alteration in the contents of P-450s that are expressed constitutively in rat livers (2–12). In our recent papers (9, 10), several hepatic P-450s have been shown to be divided into two groups, neonatal and pubertal P-450s, depending on the age of the appearance in rat livers. Constitutive expressions of neonatal P-450s such as P-450b, P-450e, P-448-H, and P-450$_{6\beta\text{-}1}$ reach their maximal levels by 4 weeks of age (7–11). At pubertal periods, their contents were rather suppressed, in accordance with the development of the growth hormone-dependent regulatory system.

In rats, a cytochrome P-450, P-450DM/j (P-450IIE) (13–16), exists and is suggested to be involved in a number of toxicologically important reactions including the metabolic activations of nitrosamines (14, 17), carbon tetrachloride (18, 19), and acetoaminophen (20). Although P-450DM/j is induced by the treatment of rats with various agents including isoniazid (13), dimethylsulfoxide (21, 22), low alkyl alcohols (21–23), ketones (14, 23), and pyrazoles (22, 24), this enzyme is detectable in low amounts in livers of untreated rats. Hepatic content of P-450DM/j is also shown to be increased in diabetic or severe fasting conditions (25, 26) and to act as a salvage function of gluconeogenesis (15, 27).

On the developmental change in the amount of P-450DM/j, the rates of microsomal aniline *p*-hydroxylation (28) and dimethylnitrosamine demethylation (22), which are mainly catalyzed by P-450DM/j, are higher in the livers of immature than adult rats. Hepatic content of this P-450 is also known to be higher in male than female rats, although P-450DM/j is detectable in the livers of both male and female rats (22). These results suggest that the hepatic level of P-450DM/j is regulated by a similar mechanism but in a manner different from other neonatal P-450s, which are regulated by growth hormone. In addition, recent studies (29) have shown that the synthesis of P-450DM/j protein is stimulated by both pre- and posttrans-

[1] P-450 isozymes P-450b, P-448-L, P-448-H, P-450e, P-450f, P-450-male, P-450-female, P-450DM/j, and PB-C correspond to P-450b, P-450c, P-450d, P-450e, P-450f, P-450h, P-450i, P-450j, and P-450k, respectively, of the terms used by Swinney *et al.* (1).

ABBREVIATIONS: P-450, cytochrome P-450; hGH, a methionylated human growth hormone; SDS, sodium dodecyl sulfate; PRL, prolactin.

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lational mechanisms in rat livers. Therefore, the effect of pituitary growth hormone on the level of both P-450DM/j mRNA and the protein has been studied. The present data clearly indicate that growth hormone suppresses the hepatic level of P-450DM/j, mainly by a pretranslational mechanism, in rats.

Experimental Procedures

Materials. Goat anti-rabbit IgG was purchased from Cappel Laboratories (Cochranville, PA), and alkaline-phosphatase-rabbit IgG was from the Binding Site Ltd (Birmingham, UK). Hydrocortisone acetate, triiodothyronine, ovine PRL, nitroblue tetrazolium, and 5-bromo-4-chloro-3-indolyl phosphate were obtained from Sigma Chemical Co. (St. Louis, MO). hGH and somatomedin C were generous gifts from Sumitomo and Fujisawa Pharmaceutical Co. (both Osaka, Japan), respectively.

Animal treatment. Sprague-Dawley rats were obtained from Clea Japan (Tokyo). Hypophysectomy was performed at 7 weeks of age (4, 5). The animals were allowed to recover for at least 1 week and were given a subcutaneous injection (2 IU/kg of body weight) twice a day or osmotic infusion (0.01 IU/h) of hGH for 7 days, as reported previously (8). PRL and somatomedin C were administered by the same method as hGH. Streptozotocin (65 mg/kg, intravenously) was given to adult 8-week-old rats. The animals showing more than 1% urinary glucose with Combistix-II (Miles-Sankyo Ltd, Tokyo) were judged to be diabetic at 3 days after the injection, and these had higher blood sugar levels of 360 to 520 mg/dl at 5 weeks after the injection. At 3 weeks after the streptozotocin injection, some diabetic rats were treated with porcine insulin (Insulatard; Nordisk Gentofte, Denmark) (12.5 IU/kg, subcutaneously) twice a day for 2 weeks. Hepatic microsomes were prepared 14–18 hr after the last dose, as previously reported (4). At the same time, some portions of livers were collected for the preparation of RNA fractions.

Immunochemical quantification. SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli (30), using 7.5% gels for the analyses. Microsomal proteins were electrophoretically transferred to nitrocellulose paper (Bio-Rad) and probed with anti-rabbit antibodies raised against P-450DM/j, which was purified from the liver of streptozotocin-induced diabetic rats (16). P-450DM/j corresponds to P-450j (1, 13), P-450$_{ac}$ (14), and RLM6 (15) as judged from the chemicophysical properties and the N-terminal amino acid sequence (16). The antibodies used did not cross-react with purified preparations of P-450b (8), P-450e (8), P-450f (31), P-450g (31), P-450-female (P-450i) (2), P-448-L (P-450c) (32), P-448-H (P-450d) (32), or P-450$_{6\beta\text{-}1/PB\text{-}1}$ (11) but weakly cross-reacted with P-450-male (P-450h) (2) and PB-C (P-450k) (7, 33). The latter two forms were, however, separable from P-450DM/j in Western blots and did not interfere with the quantitation of protein. Alkaline phosphatase activity on the immunoblots was quantitated essentially by the method of Blake *et al.* (34).

RNA blots. Total RNA was prepared by the guanidine thiocyanate method of Chirgwin *et al.* (35). The amount of RNA was determined from the absorbance at 260 nm using a Beckmann Du-65 spectrophotometer. Total RNA (20 μg) was subjected to electrophoresis at 20 V on 1.2% agarose/2.2 M formaldehyde gels and was transferred to Nytran (NY13N; Schleicher & Schuell). Filters were hybridized with a ^{32}P-labeled oligonucleotide probe in 0.5 M sodium phosphate (pH 7.2) containing 7% SDS, 1% bovine serum albumin, and 1 mM EDTA, at 42°, overnight after prehybridization with 15 mM sodium chloride/1.5 mM sodium citrate, pH 7.0 containing 0.5% SDS. The filter was washed sequentially with 0.2 M sodium phosphate (pH 7.2), 1% SDS, and 1 mM EDTA at 42° for 30 min twice, essentially following the method of Church and Gilbert (36). The filter was exposed on a X-ray film at −80°. The 23-mer oligonucleotide has a sequence of 5′-TCCAGTGACTGAAGGTGTTCCTT-3′, which is complementary to base pairs 763 to 784 of the P-450j sequence (37) and was synthesized on an Applied Biosystem 381A DNA synthesizer. The probe was 5′-end labeled with [γ-^{32}P]ATP (specific activity, 3000 Ci/mmol; Amersham) and T_4-polynucleotide kinase (Pharmacia P-L biochemicals) and purified with a NACS column (Bethesda Research, Gaithersburg, MD). The specific activity of the oligomer used for hybridization was about 5×10^6 cpm/pmol. Determination of the relative abundance of P-450DM/j mRNA was done using slot blots, similar to the method of Northern blot except that 10 μg of total RNA was used and the abundance was corrected from that of β-actin mRNA (38). The level of the hybridizable mRNA was estimated by the radioactivity on the blots.

Other assay methods. Protein concentration was determined by the method of Lowry *et al.* (39) with bovine serum albumin as the standard. Microsomal hydroxylations of *p*-nitrophenol (40) and aniline (4) were measured spectrophotometrically. A typical reaction mixture consisted of 1.0 mg of microsomal protein, 50 mM sodium/potassium phosphate (pH 7.4), 0.1 mM *p*-nitrophenol or 5 mM aniline, and an NADPH-generating system containing 0.8 mM NADP, 8 mM glucose-6-phosphate, 1 unit/ml glucose-6-phosphate dehydrogenase, and 6 mM magnesium chloride, in a final volume of 1 ml. The reaction was started by the addition of the NADPH-generating system and was incubated for 15 min at 37°. Statistical significance was determined using Student's *t* test.

Results

Effects of hypophysectomy and supplement of hGH on the content of P-450DM/j in rat livers. Hepatic content of P-450DM/j protein was quantitated by Western blots using an alkaline phosphate method. As shown in Fig. 1, P-450DM/j protein was immunostained at a slightly higher position (53 kDa) than male-dominant P-450-male (2) and PB-C (P-450k) (1, 33) (both 52 kDa) in liver microsomes of untreated rats. The specific content of P-450DM/j was increased 2.6-fold by hypophysectomy of male adult rats, and the enhanced level in hypophysectomized rats was decreased by the intermittent injection of hGH (Table 1) to mimic the secretory pattern of growth hormone in male rats (41). Hepatic content of P-450DM/j was further reduced to 40% of the intact male level by the continuous infusion of hGH, which mimicked the secretory pattern in the female rats.

The hepatic content of P-450DM/j in female rats was nearly 2-fold less than that in adult male rats but was increased by 3- to 6-fold after hypophysectomy. Therefore, no significant difference was detected in the hepatic content of P-450DM/j between male and female hypophysectomized rats. Similar to the treatment of hypophysectomized male rats, hepatic P-450DM/j content in female hypophysectomized rats was decreased by the intermittent injection of hGH to a level similar to that in intact male rats and by the continuous infusion to the level of normal female rats.

In our previous papers, both phenobarbital-inducible P-450b (8, 42) and male-dominant P-450$_{6\beta\text{-}1}$ (11, 42, 43) are shown to be suppressed by pituitary growth hormone in rats. In these experiments, contents of both P-450b and P-450$_{6\beta\text{-}1}$ in rat livers were, like P-450DM/j, increased by hypophysectomy, but their levels were almost completely suppressed by the continuous infusion of hGH (8, 11, 43).

Although hGH has both somatogenic and lactogenic binding affinities in rat livers (44), treatment of hypophysectomized male rats with ovine prolactin had no significant effect on the content of P-450DM/j (Fig. 2), indicating that the suppression of P-450DM/j is mediated through the somatogenic action of growth hormone. Somatomedin C (insulin-like growth factor

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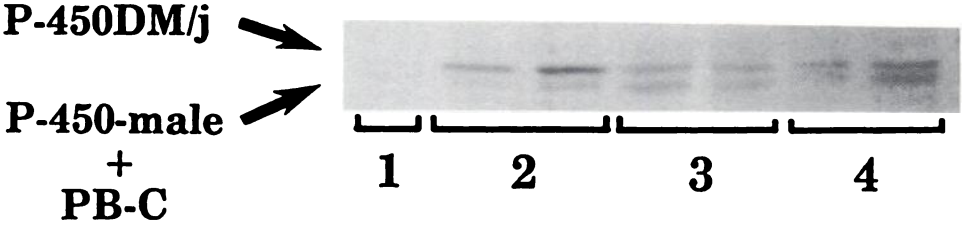

Fig. 1. Immunoblots of P-450DM/j protein in liver microsomes of intact, hypophysectomized, and hGH-treated rats. Each lane contains 10 μg of microsomal protein obtained from adult intact (*lane one*), hypophysectomized (*lane 2*), hypophysectomized and treated with hGH intermittently (*lane 3*), or hypophysectomized and infused with hGH continuously (*lane 4*) rats. Experimental details are described in Experimental Procedures.

TABLE 1

Microsomal content of P-450DM/j in livers of male and female rats

Data obtained are indicated as the mean ± standard deviation of the number of the animals indicated (*n*). Percentages relative to the corresponding control are also shown in parentheses. Hypox, hypophysectomized; GH(s), hGH-treated intermittently twice a day for 7 days; GH(i), hGH-infused by continuous osmotic infusion for 7 days.

Treatment	*n*	P-450DM/jprotein	
		Male	Female
		pmol/mg of protein	
None	3	74.8 ± 3.5 (100)	34.0 ± 5.4 (100)
Hypox	4	192.6 ± 26.7[a] (257)	189.9 ± 20.2[a] (559)
Hypox + GH(s)	4	83.3 ± 7.9 (111)	83.4 ± 7.2[a] (245)
Hypox + GH(i)	3	32.4 ± 3.8[a] (43)	37.5 ± 7.2 (110)

[a] Significantly different from the respective nontreated controls ($p < 0.05$).

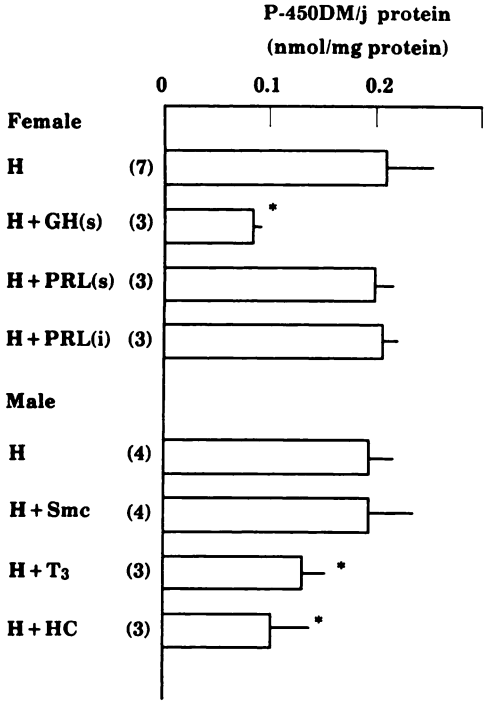

Fig. 2. Effect of PRL, somatomedin C, triiodothyronine, and hydrocortisone on the content of P-450DM/j in hypophysectomized rats. Data are shown as relative levels as compared with the hypophysectomized male and female controls. *Columns* and *bars* indicate the mean ± standard deviation of the relative values obtained from three to seven different animals as shown in parentheses. *Significantly different from the hypophysectomized group ($p < 0.05$). *H*, hypophysectomized; *GH(s)*, hGH-treated intermittently (2 IU/kg twice a day for 7 days) (i) *PRL(s)*, ovine PRL-treated intermittently (2 IU/kg twice a day for 7 days); PRL (i), ovine PRL-treated by continuous infusion (0.01 IU/hr for 7 days); *Smc*, somatomedin C-treated intermittently (100 μg/kg twice a day for 7 days); T_3 triiodothyronine-treated (50 μg/kg once a day for 7 days); *HC*, hydrocortisone acetate-treated (30 mg/kg for 3 days). Other experimental details are described in Experimental Procedures.

I), which is produced under the influence of growth hormone, has growth-promoting properties (45, 46). Therefore, somatomedin C was also given to hypophysectomized rats. However, no significant effect was observed on the hepatic contents of P-450DM/j by the administration of somatomedin C (100 μg/kg of body weight) twice a day for 7 days. Similar results were also observed with the continuous infusion (4.2 μg/hr for 7 days; data not shown). These results suggest that the suppressive effect of growth hormone is rather direct in livers and is not mediated by the production of somatomedin C. In addition, treatment of hypophysectomized rats with triiodothyronine (50 μg/kg for 7 days) or hydrocortisone acetate (30 mg/kg for 3 days) showed slight decreases in the hepatic content of P-450DM/j.

Effect of hypophysectomy and treatment with hGH on microsomal *p*-nitrophenol and aniline hydroxylations. Reinke and Moyer (40) reported that microsomal *o*-hydroxylation of *p*-nitrophenol was increased after chronic ethanol treatment of rats. In addition, this activity is also shown to be changed in parallel with the hepatic level of LM3a, an ortholog of P-450DM/j, in livers of rabbits (47).

Therefore, microsomal *p*-nitrophenol hydroxylation was chosen as a probe for confirming the change in P-450DM/j levels. As described in Table 2, microsomal hydroxylation of *p*-nitrophenol in male and female rats was increased by hypophysectomy and decreased to a level of intact male rats by the intermittent injection of hGH into hypophysectomized rats. In addition, the cathechol formation was further reduced by the continuous infusion of hGH to a level similar to that of intact female rats. Microsomal *p*-hydroxylation of aniline was also increased after hypophysectomy and was suppressed by either intermittent or continuous treatment of hGH in hypophysec-

TABLE 2

Microsomal hydroxylation of *p*-nitrophenol and aniline in hypophysectomized and growth hormone-treated rats

Microsomal activities are shown as the mean ± standard deviation of the data obtained from three to five different animals as indicated (*n*). Abbreviations used are the same as described in Table 1.

Treatment	*n*	Microsomal activities	
		p-Nitrophenol	Aniline
		nmol/mg of protein/min	
Male			
None	3	0.81 ± 0.10 (100)	0.88 ± 0.04 (100)
Hypox	5	1.19 ± 0.03[a] (146.9)	1.06 ± 0.02[a] (120.5)
Hypox + GH(s)	3	0.87 ± 0.12 (107.4)	0.71 ± 0.09[a] (80.7)
Hypox + GH(i)	3	0.45 ± 0.03[a] (55.6)	0.47 ± 0.06[a] (53.4)
Female			
None	3	0.58 ± 0.11 (100)	0.27 ± 0.02 (100)
Hypox	4	1.15 ± 0.06[a] (198.3)	0.98 ± 0.04[a] (363.0)
Hypox + GH(s)	4	0.86 ± 0.08[a] (148.3)	0.69 ± 0.04[a] (255.6)
Hypox + GH(i)	3	0.68 ± 0.09 (117.2)	0.64 ± 0.08[a] (237.0)

[a] Significantly different from the respective nontreated controls ($p < 0.05$).

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tomized rats. Williams and Simonet (48) have recently shown that microsomal demethylation of dimethylnitrosamine and content of P-450j are increased by hypophysectomy. In this paper, hepatic content of P-450j was elevated 5- to 10-fold, but the rate of the demethylation was increased less than 2-fold in hypophysectomized as compared with intact rats. The authors explain the difference as caused by the decrease in the activity of NADPH-cytochrome P-450(*c*) reductase in hypophysectomized rats. In the present study, the changes in microsomal hydroxylations of *p*-nitrophenol and aniline were largely consistent with those of the Western blots and confirmed the suppressive effect of growth hormone on hepatic P-450DM/j.

Effect of growth hormone on P-450DM/j mRNA. Acetone and isopropanol are reported to induce the hepatic content of P-450DM/j protein ($P\text{-}450_{ac}$) without increasing the level of the mRNA, but the increase in the diabetic state is reported to be the result of the increase in the level of P-450DM/j mRNA (29, 49). These results indicate that P-450DM/j protein is regulated by both a transcriptional and a translational mechanism or stabilization of protein in rat livers. To further understand the mechanism of the growth hormone-mediated control of P-450DM/j, changes in the hepatic P-450DM/j mRNA were measured using a specific oligonucleotide probe, which corresponds to a part of the nucleotide sequence (37) of P-450DM/j (base pairs 763 to 784). As shown in Fig. 3, a single hybridizing spot with the ^{32}P-labeled oligonucleotide was detected in Northern blots of hepatic RNA isolated from intact, hypophysectomized, or diabetic male rats. Changes in the relative level of P-450DM/j mRNA were also quantified with slot blots. Similar to the changes in the protein content, the hepatic level of P-450DM/j mRNA was increased 2- to 5-fold by hypophysectomy (Fig. 4). In addition, the treatment of hypophysectomized rats

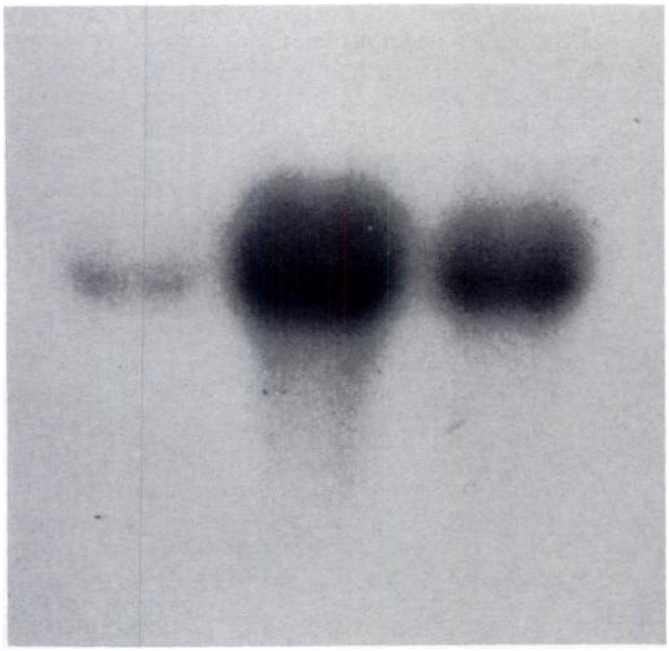

Fig. 3. Northern blot of P-450DM/j mRNA in livers of intact, hypophysectomized, and streptozotocin-induced diabetic rats. *Cont*, intact; *H*, hypophysectomized; *STZ*, streptozotocin-induced diabetic rats. Total RNA (each 20 μg) was applied for the analysis. Other experimental details are described in Experimental Procedures.

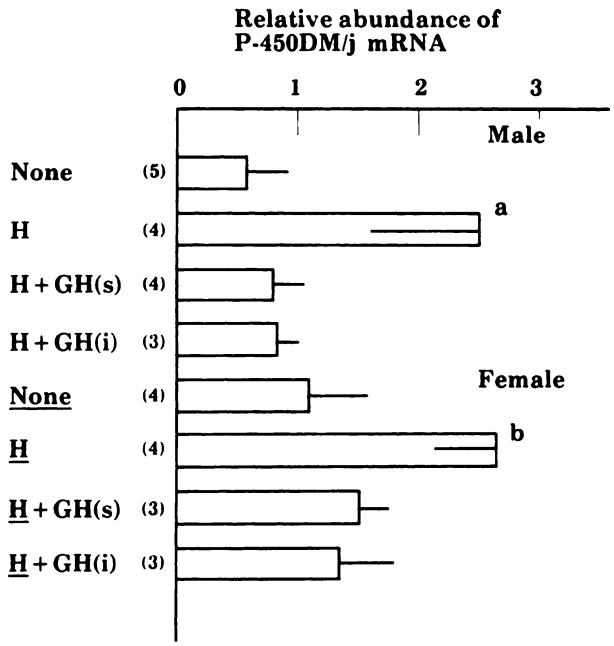

Fig. 4. Relative levels of P-450DM/j mRNA in the livers of intact, hypophysectomized, and hGH-treated rats. Abbreviations used are the same as described in Fig. 2. *Columns* and *bars* indicate the mean ± standard deviation of the data obtained from the number of animals shown in parentheses. ^{a,b}Significantly different from the nontreated male and female controls. Sex of the animals, female and male, is indicated *with* and *without underline*, respectively. The abundance of P-450DM/j mRNA was measured by slot blots and corrected from that of β-actin mRNA as described in Experimental Procedures.

with hGH suppressed the level of P-450DM/j mRNA effectively in rats infused with hGH continuously and in those treated with hGH intermittently. These results indicate that growth hormone suppresses hepatic level of P-450DM/j mainly by acting at the pretranslational step of the biosynthesis.

Comparison of hypophysectomized and diabetic states. In our recent paper (9, 10), the alteration of hepatic P-450b, $P\text{-}450_{6\beta\text{-}1}$, and P-450-male in diabetic rats has been shown to be caused mainly by the decrease in the serum growth hormone level. P-450DM/j is known to be increased in the diabetic state (14–16, 22). Thus, the levels of P-450DM/j mRNA and the protein were compared in diabetic and hypophysectomized rats. As shown in Fig. 5B, levels of P-450DM/j mRNA were largely the same between hypophysectomized and streptozotocin-induced diabetic rats. This is consistent with the idea that the changes in P-450 populations in livers of diabetic rats are mainly ascribable to the reduction of the growth hormone-mediated process (10). In contrast to the mRNA level, hepatic content of P-450DM/j protein was, however, 2.3-fold higher in diabetic rats than in hypophysectomized rats (Fig. 5A). In addition, treatment of diabetic rats with insulin effectively reduced P-450DM/j mRNA and the protein to their levels in intact rats, although similar treatment of hypophysectomized rats with insulin had no significant effect on the content of P-450DM/j protein and increased the mRNA level 3-fold.

Discussion

The present study clearly indicates the mechanism of the suppressive regulation of hepatic P-450DM/j by pituitary growth hormone. As described in Table 1, hepatic content of P-450DM/j protein was increased by hypophysectomy of male

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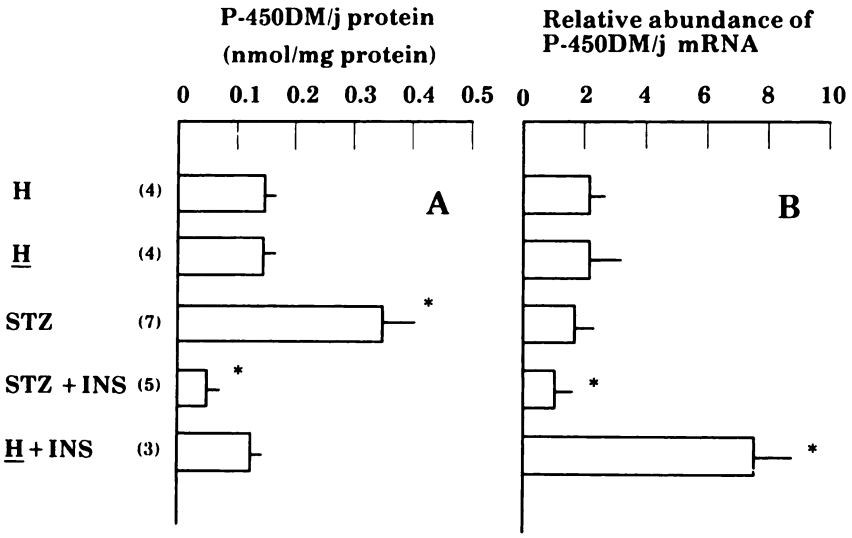

Fig. 5. Hepatic levels of P-450DM/j protein (A) and mRNA (B) in hypophysectomized and diabetic rats. *STZ*, streptozotocin-induced diabetic rats; *INS*, insulin-treated; *H*, hypophysectomized. Sex of the animals, female and male, is indicated *with* and *without underline*, respectively. *Significantly different from hypophysectomized group ($p < 0.05$). Other experimental details are the same as described in Fig. 4.

and female adult rats and was lowered by the supplement of hGH by either intermittent injection or continuous infusion, which mimicked the secretory pattern of growth hormone in male and female adult rats, respectively. In these experiments, hGH-mediated suppression was more effective in rats infused continuously than in those treated intermittently with hGH. These results are consistent with the observed sex-related difference in the hepatic content of P-450DM/j protein in rats (2-fold higher in the livers of male than female rats) (Table 1). Hepatic content of P-450DM/j is reported to be higher in the livers of immature than in adult rats (22, 23). Similar developmental profiles are also known with other hepatic P-450s, which are suppressively regulated by pituitary growth hormone (8, 9, 11). Although growth hormone is not the sole regulatory factor for the developmental changes in the amounts of hepatic P-450s, temporal changes in the hepatic level of somatogenic receptor and in serum growth hormone level could explain the developmental changes in the P-450DM/j content in rat livers as follows: similar to other neonatal P-450s, the hepatic level of P-450DM/j increases rapidly after birth (1 to 3 weeks of age), at which the level of serum growth hormone is high but the level of somatogenic receptor is negligible in the liver (50), and then the content is gradually repressed with the development of the liver somatogenic receptor and a re-increase in serum growth hormone level during pubertal periods (4 to 9 weeks of age). Consistent with this idea, P-450DM/j ($P\text{-}450_{ac}$) mRNA is reported to increase in the livers of immature rats during the maturation stage (23).

As shown in Fig. 2, PRL and somatomedin C (insulin-like growth factor I) had no significant suppressive effect on the hepatic content of P-450DM/j. Similar to the response of P-450DM/j, other growth hormone-sensitive P-450s, P-450b, P-450e, $P\text{-}450_{\beta\text{-}1}$, and P-450-male, were also refractory to the treatment with PRL or somatomedin C (8, 11, 51)[2]. These results suggest that pituitary growth hormone exerts the stimulative or suppressive effects through a common somatogenic receptor-mediated process in rat livers.

As shown in Fig. 3, the hepatic level of P-450DM/j mRNA was changed largely in parallel with the change in the protein content; the level was increased in hypophysectomized rats and decreased by the supplement of hGH to hypophysectomized rats. These results indicate that growth hormone affects P-450DM/j mainly at the pretranslational level of the activation. In this experiment, the relative level of P-450DM/j mRNA was rather higher in the liver of normal female than male rats. Similar results were also obtained with the level of the mRNA in growth hormone-treated hypophysectomized male and female rats. The reason for the apparent discrepancy between the levels of P-450DM/j protein and mRNA is unclear, but these results may imply the additional involvement of an unknown factor other than growth hormone, which also affects the hepatic level of P-450DM/j mRNA in livers of rats.

Specific mRNA for P-450DM/j is known to be elevated in the liver of diabetic rats (29, 49). In this study, the levels of P-450DM/j mRNA were largely similar to each other in the cases of hypophysectomized and diabetic rats, although the amount of P-450DM/j protein was 2.3-fold higher in diabetic than in hypophysectomized male rats. Hong *et al.* (25) reported that the level of P-450DM/j mRNA was elevated in the severely fasting state, in which the serum level of growth hormone is also low (52). These results may indicate that the main cause of the elevated level of P-450DM/j mRNA in diabetes is the depletion of growth hormone in rats. Song *et al.* (29) reported that a major component of the P-450DM/j induction response in rats is stabilization of the specific mRNA. Therefore, growth hormone may stimulate the degradation of P-450DM/j mRNA in rat livers.

In agreement with previous results (49, 53, 54), treatment of diabetic rats with insulin reversed the increased content of P-450DM/j to the levels of intact male rats. Similar treatment *in vivo* has also been shown to restore the levels of other growth hormone-susceptible P-450s in rat livers (10). Hepatocytes prepared from streptozotocin-diabetic rats were, however, reported to be resistant to the insulin effect on hepatocytes (55). Regarding the mechanism of the insulin action, we have recently shown that insulin exerts the effect on growth hormone-susceptible P-450s, P-450-male and P-450b, through the establishment of a quasi-normal hormonal state (10). In the present study, treatment of hypophysectomized rats with insulin had no significant effect on the amount of P-450DM/j protein and rather increased the mRNA (Fig. 5A). These results also support the idea that insulin mediates the normalization of hepatic

[2] Unpublished data.

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P-450s indirectly, probably through the hypothalamus-pituitary, but not directly on livers.

In our previous papers (8, 11, 43), expression of neonatal P-450s including P-450b, P-450e, and P-450$_{6\beta\text{-}1}$ in hypophysectomized rats, which were susceptible to growth hormone, were almost completely suppressed by continuous infusion of hGH. Hepatic content of P-450DM/j in hypophysectomized rats was, however, not completely diminished by the continuous infusion of hGH. In addition, the content of P-450DM/j protein, but not of the mRNA, differed between two growth hormone-depleted states in hypophysectomized and diabetic rats (Fig. 5). The reasons for these differences are unclear, but diverse chemicals such as acetone, isopropyl alcohol, and imidazole have been shown to induce hepatic P-450DM/j or the related protein without increasing the specific mRNA level (26, 29, 56). The blood levels of carbohydrate or fatty acid metabolites are also known to be elevated in the diabetic condition and have been suggested to be a cause of the induction of P-450DM/j (15, 54, 57). Therefore, an unknown component(s) derived from fatty acid and/or carbohydrate metabolism may also contribute to the enhancement of P-450DM/j protein in diabetic rats through an additional mechanism for an increase in either translation or protein stabilization. In agreement with this view, the hepatic content of P-450DM/j in hypophysectomized rats was decreased by treatment with triiodothyronine or hydrocortisone (Fig. 3), which are known to modify the carbohydrate metabolism in the liver.

In conclusion, our results indicate that pituitary growth hormone acts at a pretranslational step for the synthesis of P-450DM/j and suppresses the hepatic level in rats, although the alteration in the level of unknown translational or posttranslational agent(s) is also responsible as an additional factor on the apoprotein content of P-450DM/j in hypophysectomized and diabetic rats.

References

1. Swinney, D. C., D. E. Ryan, P. E. Thomas, and W. Levin. Regioselective progesterone hydroxylation catalyzed by eleven rat hepatic cytochrome P-450 in isozymes. *Biochemistry* **26**:7073–7083 (1987).
2. Kamataki, T., K. Maeda, Y. Yamazoe, T. Nagai, and R. Kato. Sex-difference of cytochrome P-450 in the rat: purification, characterization, and quantitation of constitutive forms of cytochrome P-450 from liver microsomes of male and female rats. *Arch. Biochem. Biophys.* **225**:758–770 (1983).
3. Kamataki, T., M. Shimada, K. Maeda, and R. Kato. Pituitary regulation of sex-specific forms of cytochrome P-450 in liver microsomes of rats. *Biochem. Biophys. Res. Commun.* **130**:1247–1253 (1985).
4. Yamazoe, Y., M. Shimada, T. Kamataki, and R. Kato. Effect of hypophysectomy and growth hormone treatment on sex-specific forms of cytochrome P-450 in relation to drug and steroid metabolisms in rat liver microsomes. *Jpn. J. Pharmacol.* **42**:371–382 (1986).
5. Kato, R., Y. Yamazoe, M. Shimada, N. Murayama, and T. Kamataki. Effect of growth hormone and ectopic transplantation of pituitary gland on sex-specific forms of cytochrome P-450 and testosterone and drug oxidations in rat liver. *J. Biochem.* **100**:895–902 (1986).
6. Morgan, E. T., C. MacGeoch, and J. A. Gustafsson. Hormonal and developmental regulation of expression of the hepatic microsomal steroid 16α-hydroxylase cytochrome P-450 apoprotein in the rat. *J. Biol. Chem.* **260**:11895–11898 (1985).
7. Waxman, D. J., G. A. Dannan, and F. P. Guengerich. Regulation of rat hepatic cytochrome P-450: age-dependent expression, hormonal imprinting and xenobiotic inducibility of sex-specific isoenzymes. *Biochemistry* **24**:4409–4417 (1985).
8. Yamazoe, Y., M. Shimada, N. Murayama, and R. Kato. Suppression of levels of phenobarbital-inducible rat liver cytochrome P-450 by pituitary hormone. *J. Biol. Chem.* **262**:7423–7428 (1987).
9. Yamazoe, Y., M. Shimada, N. Murayama, K. Nagata, and R. Kato. Hormonal regulation of hepatic cytochrome P-450, in *Xenobiotic Metabolsim and Disposition* (R. Kato, R. W. Estabrook, and M. N. Cayen, eds.). Taylor & Francis, London, 37–44 (1989).
10. 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).
11. Yamazoe, Y., N. Murayama, M. Shimada, K. Yamauchi, K. Nagata, S. Imaoka, Y. Funae, and R. Kato. A sex-specific form of cytochrome P-450 catalyzing propoxycoumarin *O*-depropylation and its identity with testosterone 6β-hydroxylase in untreated rat livers: reconstitution of the activity with microsomal lipids. *J. Biochem.* **104**:785–790 (1988).
12. Waxman, D. J., G. A. Leblanc, J. J. Morrissey, J. Staunton, and D. P. Lapenson. Adult male-specific and neonatally programmed rat hepatic P-450 forms RLM2 and 2a are not dependent on pulsatile plasma growth hormone for expression. *J. Biol. Chem.* **263**:11396–11406 (1988).
13. Ryan, D. E., L. Ramanathan, S. Iida, P. E. Thomas, M. Haniu, J. E. Shively, C. S. Lieber, and W. Levin. Characterization of a major form of rat hepatic microsomal cytochrome P-450 induced by isoniazid. *J. Biol. Chem.* **260**:6385–6393 (1985).
14. Patten, C. J., S. N. Ning, A. Y. H. Lu, and C. S. Yang. Acetone-inducible cytochrome P-450: purification, catalytic activity, and interaction with cytochrome b_5. *Arch. Biochem. Biophys.* **251**:629–638 (1986).
15. Favreau, L. V., D. M. Malchoff, J. E. Mole, and J. B. Schenkman. Responses to insulin by two forms of rat hepatic microsomal cytochrome P-450 that undergo major (RLM6) and minor (RLM6b) elevations in diabetes. *J. Biol. Chem.* **262**:14319–14326 (1987).
16. Funae, Y., S. Imaoka, and N. Shimojo. Purification and characterization of diabetes-inducible cytochrome P-450. *Biochem. Int.* **16**:503–509 (1988).
17. 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).
18. Johansson, I., and M. Ingelman-Sundberg. Carbon tetrachloride-induced lipid peroxidation dependent on an ethanol-inducible form of rabbit liver microsomal cytochrome P-450. FEBS Lett. **183**:265–269 (1985).
19. Watkins, J. B. I., R. A. Sanders, and L. V. Beck. The effect of long-term streptozotocin-induced diabetes on the hepatotoxicity of brombenzene and carbon tetrachloride and hepatic biotransformation in rats. *Toxicol. Appl. Pharmacol.* **93**:329–338 (1988).
20. Sato, C., Y. Matuda, and C. S. Lieber. Increased hepatotoxicity of acetaminophen after chronic ethanol consumption in the rat. *Gastroenterology* **80**:140–148 (1981).
21. Eliasson, E., I. Johansson, and M. Ingelman-Sundberg. Ligand-dependent maintenance of ethanol-inducible cytochrome P-450 in primary rat hepatocyte cell cultures. *Biochem. Biophys. Res. Commun.* **150**:436–443 (1988).
22. Thomas, P. E., S. Bandiera, S. L. Maines, D. E. Ryan, and W. Levin. Regulation of cytochrome P-450j, a high-affinity *N*-nitrosodimethylamine demethylase, in rat hepatic microsomes. *Biochemistry* **26**:2280–2289 (1987).
23. Hong, J. Q., J. Pan, Z. Dong, D. S. Ning, and C. S. Yang. Regulation of *N*-nitrosodimethylamine demethylase in rat liver and kidney. *Cancer Res.* **47**:5948–5953 (1987).
24. Palakodety, R. B., L. A. Clejan, G. Krikun, D. E. Feierman, and A. I. Cederbaum. Characterization and identification of a pyrazole-inducible form of cytochrome P-450. *J. Biol. Chem.* **263**:878–884 (1988).
25. Hong, J. Q., J. Pan, F. J. Gonzalez, H. V. Gelboin, and C. S. Yang. The induction of a specific form of cytochrome P-450 (P-450j) by fasting. Biochem. Biophys. Res. Commun. **142**:1077–1083 (1987).
26. Johansson, I., G. Ekström, B. Scholte, D. Puzycki, H. Jörnvall, and M. Ingelman-Sundberg. Ethanol-, fasting-, and acetone-inducible cytochromes P-450 in rat livers: regulation and characteristics of enzymes belonging to the IIB and IIE gene subfamilies. *Biochemistry* **27**:1925–1934 (1988).
27. Casazza, J. P., M. E. Felver, and R. L. Veech. The metabolism of acetone in rat. *J. Biol. Chem.* **259**:231–236 (1984).
28. El Defrawy El Masry, S., G. M. Cohen, and G. J. Mannering. Temporal changes in the microsomal drug-metabolizing system of the liver during sexual maturation. *Drug Metab. Dispos.* **2**:267–278 (1974).
29. Song, B.-J., T. Matsunaga, J. P. Hardwick, S. S. Park, R. L. Veech, C. S. Yang, H. V. Gelboin, and F. J. Gonzalez. Stabilization of cytochrome P450j messenger ribonucleic acid in the diabetic rat. *Mol. Endocrinol.* **1**:542–547 (1987).
30. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage (T_4. *Nature* (*Lond.*) **227**:680–685 (1970).
31. Bandiera, S., D. E. Ryan, W. Levin, and P. E. Thomas. Age- and sex-related expression of cytochrome P450f and P450g in rat liver. *Arch. Biochem. Biophys.* **248**:658–676 (1986).
32. Kamataki, T., K. Maeda, Y. Yamazoe, N. Matsuda, K. Ishii, and R. Kato. A high-spin form of cytochrome P-450 highly purified from polychlorinated biphenyl-treated rats. *Mol. Pharmacol.* **24**:146–155 (1983).
33. Larrey, D., L. M. Distlerath, G. A. Dannan, G. R. Wilkinson, and F. P. Guengerich. Purification and characterization of the rat liver microsomal cytochromes P-450 involved in the 4-hydroxylation of debrisoquine, a prototype of genetic variation in oxidative drug metabolism. *Biochemistry* **23**:2787–2795 (1984).
34. Blake, M. S., K. H. Johnston, G. J. Russell-Jones, and E. C. Gotschlich. A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. *Anal. Biochem.* **136**:175–179 (1984).
35. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. Isolation of biological active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**:5294–5299 (1979).
36. Church, G. M., and W. Gilbert. Genomic sequencing. *Proc. Natl. Acad. Sci. USA* **81**:1991–1995 (1984).

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37. Song, B.-J., H. V. Gelboin, S. S. Park, C. S. Yang, and F. J. Gonzalez. Complementary DNA and protein sequences of ethanol-inducible rat and human cytochrome P-450s. *J. Biol. Chem.* **261**:16689–16697 (1986).
38. Shimada, M., N. Murayama, K. Yamauchi, Y. Yamazoe, and R. Kato. Suppression in the expression of a male-specific cytochrome P-450, P-450-male: difference in the effect of chemical inducers on P450-male mRNA and protein in rat livers. *Arch. Biochem. Biophys.* **270**:578–587 (1989).
39. Lowry, O. H., A. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
40. Reinke, L. A., and M. J. Moyer. *p*-Nitrophenol hydroxylation: a microsomal oxidation which is highly inducible by ethanol. *Drug Metab. Dispos.* **13**:548–552 (1985).
41. Eden, S. Age- and sex-related differences in episodic growth hormone secretion in the rat. *Endocrinology* **105**:555–560 (1979).
42. Yamazoe, Y., M. Shimada, K. Yamauchi, N. Murayama, and R. Kato. Alteration of hepatic drug metabolizing activities and contents of cytochrome P-450 isozymes by neonatal monosodium glutamate treatment. *Biochem. Pharmacol.* **37**:1687–1691 (1988).
43. Yamazoe, Y., M. Shimada, N. Murayama, S. Kawano, and R. Kato. The regulation by growth hormone of microsomal testosterone 6β-hydroxylase in male livers. *J. Biochem.* **100**:1095–1097 (1986).
44. Norstedt, G., G. Andersson, and J. Å. Gustafsson. Growth hormone induction of lactogenic receptors at intracellular sites in male rat liver. *Endocrinology* **115**:672–680 (1984).
45. Phillips, L. S., and R. Vassilopoulos-Sellin. Somatomedins. *N. Engl. J. Med.* **302**:371–380 (1980).
46. Jansen, M., F. M. A. van Schaik, A. T. Ricker, B. Bullock, D. E. Woods, K. H. Gabbay, A. L. Nussbaum, J. S. Sussenbach, and J. L. Van den Brande. Sequence of cDNA encoding human insulin-like growth factor I precursor. *Nature* (*Lond.*) **306**:609–611 (1983).
47. Koop, D. R. Hydroxylation of *p*-nitrophenol by rabbit ethanol-inducible cytochrome P-450 isozyme 3a. Mol. Pharmacol. **29**:399–404 (1986).
48. Williams, M. T., and L. C. Simonet. Effects of growth hormone on cytochrome P-450j. *Biochem. Biophys. Res. Commun.* **155**:392–397 (1988).
49. 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 P-450ac (p-450j) in chemically induced and spontaneously diabetic rats. *Arch. Biochem. Biophys.* **263**:29–35 (1988).
50. Maes, M., R. de Hertogh, P. Watrin-Granger, and J. M. Ketelslegers. Ontogeny of liver somatotropic and lactogenic binding sites in male and female rats. *Endocrinology* **113**:1325–1332 (1983).
51. Guzelian, P., S. D. Li, E. G. Schuetz, P. Thomas, W. Levin, A. Mode, and J. A. Gustafsson. Sex change in cytochrome P-450 phenotype by growth hormone treatment of adult rat hepatocytes maintained in a culture system on matrigel. *Proc. Natl. Acad. Sci. USA* **85**:9783–9787 (1988).
52. Tannenbaum, G. S., O. Rorstad, and P. Brazeau. Effects of prolonged food deprivation on the ultradian growth hormone rhythm and immunoreactive somatostatin tissue levels in the rat. *Endocrinology* **104**:1733–1738 (1979).
53. Favreau, L. V., and J. B. Schenkman. Composition changes in hepatic microsomal cytochrome P-450 during onset of streptozotocin-induced diabetes and during insulin treatment. *Diabetes* **37**:577–584 (1988).
54. Bellward, G. D., T. Chang, B. Rodrigues, J. H. McNeill, S. Maines, D. E. Ryan, W. Levin, and P. E. Thomas. Hepatic cytochrome P-450j induction in the spontaneously diabetic BB rat. *Mol. Pharmacol.* **33**:140–143 (1988).
55. Hussin, A. H., and P. Skett. Lack of effect of insulin in hepatocytes isolated from streptozotocin-diabetic male rats. *Biochem. Pharmacol.* **37**:1683–1686 (1988).
56. Khani, S. C., P. G. Zaphiropoulos, V. S. Fujita, T. D. Porter, D. R. Koop, and M. J. Coon. cDNA and derived amino acid sequence of ethanol-inducible rabbit liver cytochrome P-450 isozyme 3a (P-450ALC). *Proc. Natl. Acad. Sci. USA* **84**:638–642 (1987).
57. Tu, Y. Y., and C. S. Yang. High-affinity nitrosamine dealkylase system in rat liver microsomes and its induction by fasting. *Cancer Res.* **43**:623–629 (1983).

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