Regulations of Male-Dominant P-450Md mRNA in Rat Liver by Hormonal Factors and Xenobiotics

Kiyoshi Nagata, Miki Shimada, Yasushi Yamazoe and Ryuichi Kato

Department of Pharmacology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan

Received June 10, 1991 Accepted July 22, 1991

ABSTRACT — Male-dominant P-450Md mRNA was undetectable in the livers of newborn rats. In female rat livers, the mRNA appeared at 17 days of age and then decreased to very low levels in the adult periods. The level of P-450Md mRNA in female rats was increased by phenobarbital or dexamethasone treatment, whereas the level in the males was depressed by methylcholanthrene. Hypophysectomy decreased the level of P-450Md mRNA in male rat livers, and continuous infusion or twice-daily injections of growth hormone to hypophysectomized rats caused further suppression or clear restoration, respectively, of the mRNA level.

More than 10 different forms of cytochrome P-450 are contained in hepatic microsomes, and these enzymes catalyze the oxidative metabolism of a wide variety of xenobiotic and endogenous substrates. The contents of most cytochrome P-450 forms in rat livers are sex-related: male- or female-specific and male- or female-dominant (1–7). These sex-related differences are generally related to the differences in the level of their mRNAs (6–9). Previously, we isolated and sequenced a new cytochrome P-450 cDNA, designated as P-450Md cDNA (EMBL accession no. X53477), from a gtll library constructed from male rat livers by using P-450-male cDNA (1, 10).

More recently, Emi et al. (11) have reported the appearance of a 49-KDa protein (P-49) during primary culture of male rat hepatocytes. To characterize P-49, they isolated cDNA encoding P-49 and sequenced it. The sequence of P-49 cDNA has high homology to that of P-450Md cDNA, but is different in 6 nucleotides in the coding region, causing 5 amino acid substitutions (10, 11).

The levels of both P-450Md mRNA and P-49 mRNA were undetectable in newborn rats and appeared in adult rats with male dominancy (10, 11). These results suggest that P-450Md mRNA and P-49 mRNA are transcriptional products of a similar or allelic gene.

Since the expressions of many cytochrome P-450 are regulated by sex-hormones, growth hormone and thyroid hormone (6, 7, 12, 13), we examined hormonal regulation of the expression of P-450Md mRNA in rat livers.

Male and female Sprague-Dawley rats were obtained from Clea Japan (Tokyo). Hypophysectomy was performed at 7 weeks of age and 2 weeks before the experiment (2, 3). Human growth hormone was kindly supplied by Sumitomo Pharmaceutical Co., Ltd. (Osaka). Total RNAs were isolated from rat livers, and the P-450Md mRNA level was determined by Northern blot analysis of 2.2 M formaldehyde gels by using 32P-labeled specific oligonucleotide probes as described previously (8, 9). Highly specific sequences in the noncoding region (1575 bp to 1604 bp) and the coding re-
gion (1090 bp to 1123 bp) were selected, and 30-mer (5'-ACAGTTCAGAGGTAGCATCC-CTGCCATTCA-3') and 34-mer (5'-TCCACATCCTGCGTTGTCTTACGAGGGA-A-3') oligonucleotides, which are complementary to P-450Md mRNA, were synthesized (9, 10).

As shown in Fig. 1, P-450Md mRNA was undetectable in the livers of newborn rats and 3 and 7 day-old rats. In female rat livers, the mRNA (1.9 kb) appeared clearly at 17 days and then decreased to very low levels in 9 weeks and 9 months. On the other hand, in male rat livers, P-450Md mRNA also appeared in 17 days and then increased to a high adult level in 9 weeks. These expression and suppression patterns of P-450Md mRNA in the livers of female and male rats are partially similar, but are not identical to those of mRNAs encoding P-450-male (11C11) and P-450-female (IIC12) and P-450fl (IIIA2) (1, 8).

The level of P-450Md mRNA in male rat livers was not changed by phenobarbital treatment, but the level in female rats was markedly increased by phenobarbital, dexamethasone or pregnenolone-16α-carbonitrile treatment (Fig. 2). The level of P-450Md is known to be expressed in female rat livers by phenobarbital, dexamethasone or pregnenolone-16α-carbonitrile treatment (8). On the other hand, the level of P-450Md mRNA in male rat livers was markedly decreased by 3-methylcholanthrene treatment. A similar decrease in the level of P-450-male mRNA in male rat livers by methylcholanthrene treatment was reported (9).

It has been reported that hypophysectomy of male rats markedly decreases the hepatic level of P-450-male mRNA (6–9). The level of P-450Md mRNA in male rat livers was also markedly decreased by hypophysectomy (Fig. 2). Treatment with growth hormone (2 IU/kg) subcutaneously for 7 days, which mimics the male pattern of growth hormone secretion, markedly stimulated the expression of P-450Md mRNA, whereas continuous injection of growth hormone (0.01 IU/hr) for 7 days using an Alza mini-pump (2), which mimics the female pattern of secretion, further suppressed the expression of P-450Md mRNA (Fig. 2). This mode of action of growth hormone was similar in P-450 male mRNA and P-450Md mRNA, indicating that the expression of both mRNAs is regulated by a closely related mechanism (6, 9, 14). The regulations in the expression of P-450Md mRNA by growth hormone differ from those of P-450fl in their response to the intermittent injection of growth hormone, but is similar to the regulations of P-450f (11C7) (8). The response of P-450Md
mRNA to continuous injection of growth hormone is, however, not identical to that of P450f (14).

Moreover, we and others have recently reported that treatment of hypophysectomized rats with thyroid hormone markedly decreased the expressions of P-450b (IIB1), P-450e (IIB2), P-450b, and P-448-H (IA2) (12, 13, 15). However, the same treatment with thyroid hormone (T₃, 50 µg/kg, s.c., 7 days) or 3-methylcholanthrene (MC, 40 mg/kg for 3 days) and liver total RNAs were prepared 24 hr after the last treatments. (b) Adult male rats were hypophysectomized, and growth hormone (GH) was given subcutaneously (s) or by continuous infusion (i) as described previously (2).

**Fig. 2.** Effects of various inducers and growth hormone on the hepatic P-450Md mRNA level in normal and hypophysectomized rats. Northern blotting was done with 20 µg of total RNA for each sample using the oligonucleotide probe of the noncoding region, and similar results were obtained with the coding region probe. (a) Adult male and female rats were intraperitoneally treated with phenobarbital (PB, 80 mg/kg for 3 days), pregnenolone-16α-carbonitrile (PCN, 75 mg/kg for 3 days), dexamethasone (Dex, 100 mg/kg for 4 days) or 3-methylcholanthrene (MC, 40 mg/kg for 3 days); and liver total RNAs were prepared 24 hr after the last treatments. (b) Adult male rats were hypophysectomized, and growth hormone (GH) was given subcutaneously (s) or by continuous infusion (i) as described previously (2).

These results indicate that the expression of the mRNA encoding sex-related cytochrome P-450 is mainly regulated by the mode of growth hormone secretion, but other factors, such as thyroid hormone, may modulate the action of growth hormone (12, 13, 15).

**REFERENCES**

1. Kamataki, T., Maeda, K., Yamazoe, Y., Nagai, T. and Kato, R.: 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)

2. Yamazoe, Y., Shimada, M., Kamataki, T. and Kato, R.: 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. Japan. J. Pharmacol. 42, 371–382 (1986)

3. Kato, R., Yamazoe, Y., Shimada, M., Murayama, N. and Kamataki, T.: 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)

4. Yamazoe, Y., Shimada, M., Murayama, N. and Kato, R.: Suppression of levels of phenobarbital-inducible rat liver cytochrome P-450 by pituitary hormone. J. Biol. Chem. 262, 7423–7428 (1987)
5 Yamazoe, Y., Shimada, M., Murayama, N., Kawano, S. and Kato, R.: The regulation by growth hormone of microsomal testosterone 6β-hydroxylase in male rat livers. J. Biochem. 100, 1095–1097 (1986)
6 Mode, A., Wiersma-Larsson, E., Ström, A., Zaphiropoulos P.G. and Gustafsson, J.-Å.: A dual role of growth hormone as a feminizing and masculinizing factor in the control of sex-specific cytochrome P-450 isozymes in rat liver. J. Endocrinol. 120, 311–317 (1989)
7 Janeczko, R., Waxman, D.J., Le Blanc, G.A., Morville, A. and Adesnik, M.: Hormonal regulation of levels of the messenger RNA encoding hepatic P-450 2c (IIC11), a constitutive male-specific form of cytochrome P-450. Mol. Endocrinol. 4, 295–303 (1990)
8 Shimada, M., Nagata, K., Murayama, N., Yamazoe, Y. and Kato, R.: Role of growth hormone in modulating the constitutive and phenobarbital-induced levels of two P-450α (testosterone 6β-hydroxylase) mRNAs in rat livers. J. Biochem. 106, 1030–1034 (1989)
9 Shimada, M., Murayama, N., Yamauchi, K., Yamazoe, Y. and Kato, R.: Suppression of hepatic levels of phenobarbital-inducible P-450b and P-450e, and other neonatal P-450s in hypophysectomized rats by thyroid hormone. Biochem. Biophys. Res. Commun. 160, 609–614 (1989)
10 Nagata, K., Sasamura, H., Miyata, M., Shimada, M., Yamazoe, Y. and Kato, R.: Suppression in the expression of a male-specific cytochrome P-450, P-450f mRNA: a new mode of regulation. Mol. Cell. Endocrinol. 68, 53–60 (1990)
11 Emi, Y., Chijiwa, C. and Omura, T.: A different cytochrome P-450 form is induced in primary cultures of rat hepatocytes. Proc. Natl. Acad. Sci. U.S.A. 87, 9746–9750 (1990)
12 Yamazoe, Y., Murayama, N., Shimada, M. and Kato, R.: Suppression of hepatic levels of phenobarbital-inducible P-450b and P-450e, and other neonatal P-450s in hypophysectomized rats by thyroid hormone. Biochem. Biophys. Res. Commun. 160, 609–614 (1989)
13 Murayama, N., Shimada, M., Yamazoe, Y. and Kato, R.: Difference in the susceptibility of two phenobarbital-inducible forms, P450IIIB1 and P450IIIB2, to thyroid hormone- and growth hormone-induced suppression in rat liver: phenobarbital-inducible P450IIIB2 suppression by thyroid hormone acting directly, but not through the pituitary system. Mol. Pharmacol. 39, 811–817 (1991)
14 Sasamura, H., Nagata, K., Yamazoe, Y., Shimada, M., Saruta, T. and Kato, R.: Effect of growth hormone on rat hepatic cytochrome P-450f mRNA: a new mode of regulation. Mol. Cell. Endocrinol. 68, 53–60 (1990)
15 Waxman, D.J., Ram, P.A., Notani, G., LeBlanc, G.A., Alberta, J.A., Morrissey, J.J. and Sundseth, S.S.: Pituitary regulation of the male-specific steroid 6β-hydroxylase P-450 2a (gene product II/A2) in adult rat liver. Suppressive influence of growth hormone and thyroxine acting at a pretranslational level. Mol. Endocrinol. 4, 447–454 (1990)