BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **242,** 57–60 (1998)
ARTICLE NO. RC977904

Sexually Dimorphic Expression of Rat CYP3A9 and CYP3A18 Genes Is Regulated by Growth Hormone

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Received November 4, 1997

The cDNAs for two CYP3A genes were isolated from the livers of rats using an RT-PCR approach with CYP3A subfamily-specific primers. Sequence analysis revealed these cDNAs to be identical to CYP3A9, which had previously been isolated from rat brain and nasal epithelium and the recently described CYP3A18. The hepatic expression of both genes was sexually dimorphic. Thus CYP3A18 mRNA levels were 25-fold higher in male livers compared to females, while CYP3A9 showed a reverse pattern with 6-fold higher expression in the liver of females. Exposure of male rats to the female pattern of growth hormone secretion led to an increase in hepatic CYP3A9 mRNA expression and suppressed expression of CYP3A18. These findings indicate that the CYP3A subfamily in rats has both male- and female-specific isoforms which are regulated by growth hormone in a manner similar to some other sexually dimorphic cytochrome P450s.

Key Words: **cytochrome P450; gender-dependent expression; hepatic regulation; growth hormone.**

The cytochrome P450 (P450) superfamily (1) is a large group of heme-containing microsomal proteins that catalyse the oxidation of diverse endogenous and foreign substrates. In mammalian liver, members of the CYP3A subfamily are among the most abundant P450 proteins, being involved in the metabolism of such physiologically important compounds as bile acids (2), steroid hormones (3-5) and retinoic acid (6), as well catalyzing the oxidation of many procarcinogens and pharmaceutical agents (7-12). Currently, the cDNAs of five members of the rat CYP3A subfamily have been characterized; CYP3A1 (13), CYP3A2 (14), CYP3A9 (15,16), CYP3A18 (17,18) and CYP3A23 (19,20). While most studies have concentrated on defining the expression of CYP3A genes in liver (13,16-21), they have also been detected in other tissues including brain (15), intestine (22) and leucocytes (23).

Mammalian hepatic CYP3A genes are highly inducible by reagents such as clotrimazole, pregnenolone-16α-carbonitrile, phenobarbital and dexamethasone (16,17,19,24,25). Another characteristic feature of the physiological regulation of hepatic CYP3A genes is age- and sex-dependent expression. For instance, CYP3A2 is expressed in the livers of both female and male neonatal rats, but levels decline to undetectable levels at puberty in females (14,16), whereas they are preserved throughout life in males. In contrast, CYP3A9 is absent until puberty, when its hepatic expression increases to a higher level in females than in males (16). CYP3A18 is expressed predominantly in male rats (17), a similar pattern to that exhibited by the hamster CYP3A10 gene which encodes lithocholic acid 6β-hydroxylase (2,26).

Sexual dimorphism in the expression of many rodent hepatic genes, including CYP genes (27), is primarily mediated via the gender-specific profile of pituitary growth hormone secretion. Adult males have a pulsatile profile, with peaks of high concentrations (200-300 ng/ml) of circulating growth hormone every 3-4 hr; between these peaks growth hormone declines to undetectable levels. In females, serum levels of growth hormone are lower and the period between pulses is shorter, resulting in a relatively uniform concentration of 10 to 20 ng/ml.

We have previously observed an increase in lithocholic acid 6β-hydroxylase activity in the livers of bile-duct-ligated rats (Jiezhong Chen and GCF, unpublished). The present studies were initiated in an attempt to identify and characterize the rat CYP gene(s) responsible for this induction. Because none of the rat CYP3A enzymes have been shown to be active in bile acid hydroxylation, we used RT-PCR with CYP3A subfamily-specific primers to identify novel CYP3A genes

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Abbreviations: P450, cytochrome P450; RT-PCR, reverse transcriptase-polymerase chain reaction; RACE, rapid amplification of cDNA ends; 5′-UTR, 5′-untranslated region.

0006-291X/98 $25.00

expressed in the livers of rats that had been subjected to bile duct ligation. Two cDNAs corresponding to the recently described CYP3A9 and CYP3A18 genes were isolated and found to be differentially regulated by growth hormone in male and female rats.

MATERIALS AND METHODS

Materials. [α-^{32}P]dCTP (3000 Ci/mmol) and Hybond N$^+$ membranes were purchased from Amersham (Amersham, UK): recombinant human growth hormone from Pharmacia Upjohn (Sydney, Australia): terminal deoxynucleotidyl transferase from Promega (Madison, WI): oligonucleotides from BRESATEC (Adelaide, Australia) and restriction enzymes from Boerhinger-Mannheim (Mannheim, Germany)

Animals. Wistar rats were housed in the Westmead Hospital Animal Care Department and were treated according to research protocols approved by the Westmead Hospital Animal Care and Ethics Committee. Bile duct ligation was performed according to a previously reported protocol (28). Animals were allowed to recover for 5 days before tissues were removed under ether anaesthesia, snap frozen in liquid nitrogen and stored at −80°C for subsequent analysis. Recombinant human growth hormone (3.0 IU/mg) was administered to male rats via subcutaneous osmotic minipumps (Alza; Palo Alto, CA) at a rate of 20 μg/hr/kg for 7 days. Castration was performed in male rats as described previously from this laboratory (29).

cDNA cloning and sequence analysis. Partial cDNAs for novel CYP3A genes were obtained from total liver RNA by RT-PCR using oligonucleotide primers based on the most highly conserved regions of previously characterized CYP3A genes. The sequences of these primers: 5′-GCACATCATTTGGAGTGAA-3′ and 5′-ACCTCATGTCAATGCAGT-3′ correspond to nucleotides 623-641 and 1408-1391 respectively of the rat CYP3A2 cDNA (14). PCR products were cloned into the pGEM-T vector (Promega) and sequenced in both directions on an automated DNA sequencer (Applied Biosystems, model 373A). Complete cDNAs were synthesized by a modified RACE protocol (30) using the Marathon kit (Clontech; Palo Alto, CA).

RNA isolation and Northern analysis. Total RNA was purified from rat tissues by the guanidinium/CsCl method (31) or RNeasy columns (Qiagen; Hilden, Germany). Ten micrograms of total RNA were electrophoresed on 1% agarose gels and transferred to Hybond N$^+$ membranes. CYP3A9 and CYP3A18 specific oligonucleotide probes corresponding to nucleotides 1946-1925 (16) and 1729-1708 (17) respectively of the 3′ non-translated regions were end-labelled by the terminal nucleotidyl transferase reaction and hybridized to filters. The membranes were stripped and probed with an 18S rRNA oligonucleotide as an internal standard for equivalence of loading. Bands were visualized by autoradiography, and the intensities of bands were quantified using an image analysis system (Molecular Dynamics; Sunnyvale, CA).

RESULTS AND DISCUSSION

Sequence analysis of the 785 bp products derived from RT-PCR with primers specific for the CYP3A subfamily revealed sequences corresponding to the previously characterized 3A2 and 3A23 genes, as well as two related but clearly distinct partial cDNAs. The complete sequence of one of these cDNAs was identical to CYP3A18, a gene that has previously been characterized in rat liver (17,18). The sequence of the other cDNA was identical to that of CYP3A9 which was recently isolated from rat brain (15), except for the length

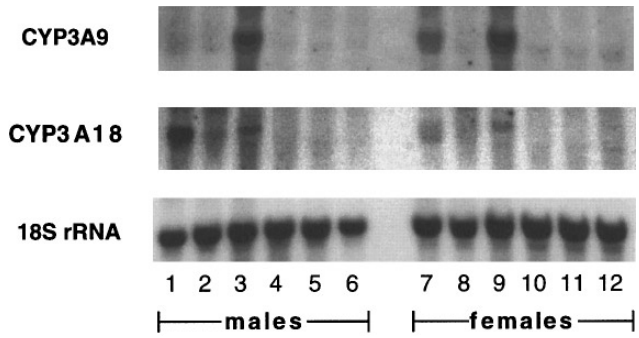

FIG. 1. Northern analysis of CYP3A9 and CYP3A18 expression in several tissues of adult male (lanes 1–6) and female rats (lanes 7–12). Total RNA was isolated from liver (lanes 1 and 7), brain (lanes 2 and 8), small intestine (lanes 3 and 9), kidney (lanes 4 and 10), spleen (lanes 5 and 11) and lung (lanes 6 and 12) and probed sequentially with oligonucleotides specific for the 3′ untranslated regions of CYP3A9 (5′-CTTGCACAGAGGCTGAGAAAGC-3′) and CYP3A18 (5′-AGCCACTGAGCCTGTTCAGTGA-3′) genes. The Northern transfer was then stripped and reprobed with an 18S rRNA oligonucleotide to assess equivalent loading.

of the 5′-untranslated region (5′-UTR). This region was 87 bp compared with 279 bp for the CYP3A9 cDNA isolated from rat brain (15). A shorter 5′-UTR is consistent with the length of other rat CYP3A genes expressed in the liver and, in particular, the mouse CYP3A13 (32) which has 91.6% nucleotide identity with CYP3A9. The present findings are therefore consistent with the possibility that the CYP3A9 gene may have alternative transcription start sites when expressed in the liver and brain. The alternative possibility that we did not obtain the full length cDNA by the RACE protocol is unlikely because a CYP3A9 cDNA isolated from rat olfactory epithelium also had a shorter 5′-UTR of 65bp (16).

The expression of the CYP3A9 and CYP3A18 genes in several tissues of male and female rats was examined by Northern analysis (Fig. 1). Both genes were expressed predominantly in the liver and small intestine. Very low levels of mRNA were detectable in brain, kidney, lung and spleen after prolonged exposure of the filters. In the small intestine, the levels of CYP3A18 were comparable in males and females. However, as also reported recently by others (17), hepatic expression of CYP3A18 mRNA was at much higher levels in male than in female rats. In contrast, CYP3A9 mRNA was more abundant in female livers than in males. This finding constitutes the first example of a CYP3A gene with a female-predominant pattern of hepatic expression. Such gender-dependent expression reinforces the similarities in the regulation of genes from the rat CYP3A subfamily with other CYP subfamilies, such as the CYP2C subfamily which have both male and female-specific isoforms (33,34).

Quantification of the abundance of CYP3A9 mRNA (Fig. 2) showed that CYP3A9 was expressed at 6-fold

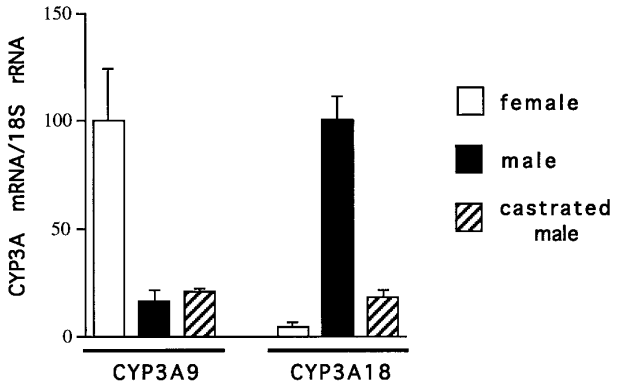

FIG. 2. Comparison of the abundance of CYP3A9 and CYP3A18 mRNA in the livers of female, male and castrated male rats. The data are the mean and standard error for 3 rats and are expressed as a percentage of the level of CYP3A9 in females and of CYP3A18 in males. Expression of the CYP3A9 gene was significantly lower ($p<0.05$) in intact and castrated male rats compared with females, while the level of CYP3A18 mRNA was greater in intact male versus female and castrated male rats ($p<0.005$).

higher levels in female than in male rat livers. This is a more extreme pattern of sexually-dimorphic expression than the two-fold difference described previously (16); the reason is not clear but may represent differences between rat strains. The female-predominant expression of CYP3A9 is similar to that observed for CYP2C7 which is 4-fold higher in female rats than in males, but contrasts with that of the female-specific CYP2C12 which is usually undetectable in males (27,34,35).

The levels of CYP3A18 mRNA in male livers were 25 times those observed in females. This resembles the 50-fold difference in CYP3A10 levels that occurs between male and female hamsters. This analogous expression, together with the high degree of sequence similarity supports the concept that CYP3A18 could be the rat orthologue of the hamster lithocholic acid 6β-hydroxylase (2,17,26). CYP3A18 and CYP3A10 are more similar to each other than to other CYP3A genes; they share 85% nucleotide identity (17). There is a discrepancy between the expression pattern observed for CYP3A18 in the present and in a previous study (16) in which no difference between males and females was observed from birth to 20 weeks. The reason for this is not known, but again it may be due to the different rat strains used in the two studies.

To determine whether the gender-dependent expression of the CYP3A9 and 3A18 genes was influenced by the presence of circulating gonadal androgens in males, the abundance of CYP3A9 and 3A18 mRNAs were examined in the livers of castrated male rats (Fig. 2). CYP3A9 levels were the same in castrated and intact males, indicating that the female-predominant expression of CYP3A9 could not be due to suppression by testosterone in males. On the other hand, CYP3A18 expression was reduced in castrated males almost to female levels. Thus the higher levels of CYP3A18 mRNA in male livers are regulated at least in part, either directly or indirectly, by gonadal androgens.

The sex-dependent profile of circulating growth hormone has been shown to be responsible for the sexually-dimorphic expression of many hepatic genes including members of the CYP2A, 2C and 3A subfamilies. In female rats, pituitary secretion of growth hormone is characterized by a high pulse frequency that results in continuous circulating levels of growth hormone. In male rats, there are less frequent but regular peaks every 3-4 hours interspersed by periods when serum growth hormone levels are undetectable. It has been established that a uniform level of as little as 3% of the normal female growth hormone concentration in hypophysectomized rats is sufficient to essentially 'feminise' rats, thereby completely suppressing the expression of male-dependent P450 isoforms (27).

To determine whether growth hormone is involved in the regulation of the female-predominant CYP3A9 and male-specific CYP3A18, male rats were continuously infused with growth hormone delivered via a subcutaneous osmotic minipump. Such administration of growth hormone increased hepatic CYP3A9 mRNA to a level intermediate between untreated males and females (Fig. 3). At the same time, CYP3A18 mRNA was reduced to undetectable levels. These findings indicate that the regulation of CYP3A18 in male rats is similar to other male-specific P450s which are completely suppressed by the continuous, feminine profile of growth hormone secretion (27,34,36). This mode of regulation reinforces the similarity between CYP3A18 and the hamster CYP3A10 referred to earlier; the 50-fold higher expression of CYP3A10 in the liver of male hamsters (2) is mediated by growth hormone signalling via

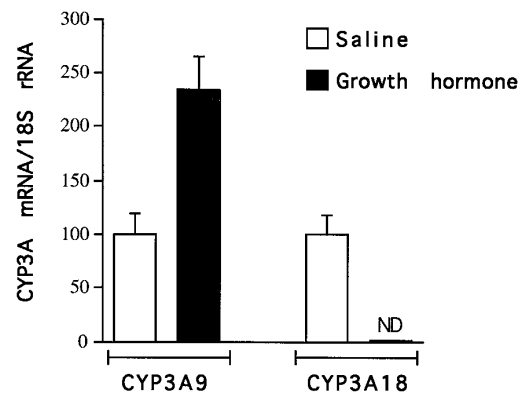

FIG. 3. Hepatic expression of the CYP3A9 and CYP3A18 genes in male rats with a 'feminized' pattern of growth hormone secretion. Male rats were implanted with minipumps which continuously delivered growth hormone or saline for 7 days. The levels of CYP3A9 mRNA were increased, while expression of the male-specific CYP3A18 gene was undetectable after growth hormone treatment. The data are the mean and standard error of 4 rats for each group. ND, not detected.

STAT transcription factors (37). It will now be of interest to characterize the substrate specificity of the rat CYP3A18 with regard to steroid and bile acid hydroxylation to determine whether it performs in rats an analogous function to the hamster CYP3A10.

ACKNOWLEDGMENTS

This work was supported by project grant 96804 from the Australian National Health and Medical Research Council and the Robert W. Storr bequest of the Medical Foundation, University of Sydney. We thank Bryan Goodwin for many helpful discussions and Jacqueline Field for valuable technical assistance.

REFERENCES

1. Nelson, D. R., Koymans, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D. J., Waterman, M. R., Gotoh, O., Coon, M. J., Estabrook, R. W., Gunsalus, I. C., and Nebert, D. W. (1996) *Pharmacogenetics* **6,** 1–42.
2. Teixeira, J., and Gil, G. (1991) *J. Biol. Chem.* **266,** 21030–21036.
3. Gonzalez, F. J. (1989) *Pharmacol. Rev.* **40,** 243–288.
4. Guengerich, F. P. (1992) *FASEB J.* **6,** 745–748.
5. Chang, T. K. H., Teixeira, J., Gil, G., and Waxman, D. J. (1993) *Biochem. J.* **291,** 429–434.
6. Martini, R., and Murray, M. (1993) *Arch. Biochem. Biophys.* **303,** 57–66.
7. Brian, W. R., Sari, M.-A., Iwasaki, M., Shimada, T., Kaminsky, L. S., and Guengerich, F. P. (1990) *Biochemistry* **29,** 11280–11292.
8. Guengerich, F. P. (1992) *Pharmac. Ther.* **54,** 17–61.
9. Gonzalez, F. J., and Gelboim, H. V. (1994) *Drug Metab. Rev.* **26,** 165–183.
10. Wrighton, S. A., Brian, W. R., Sari, M.-A., Iwasaki, M., Guengerich, F. P., Raucy, J. L., Molowa, D. T., and Vandenbranden, M. (1990) *Mol. Pharmacol.* **38,** 207–213.
11. Lemoine, A., Gautier, J. C., Azoulay, D., Kiffel, L., Belloc, C., Guengerich, F. P., Maurel, P., Beaune, P., and Leroux, J. P. (1993) *Mol. Pharmacol.* **43,** 827–832.
12. Cresteil, T., Monsarrat, B., Alvinerie, P., Treluyer, J. M., Vieira, I., and Wright, M. (1994) *Cancer Res.* **54,** 386–392.
13. Gonzalez, F. J., Nebert, D. W., Hardwick, J. P., and Kasper, C. B. (1985) *J. Biol. Chem.* **260,** 7435–7441.
14. Gonzalez, F. J., Song, B-J., and Hardwick, J. P. (1986) *Mol. Cell. Biol.* **6,** 2969–2976.
15. Wang, H., Kawashima, H., and Strobel, H. W. (1996) *Biochem. Biophys. Res. Commun.* **221,** 157–162.
16. Mahnke, A., Strotkamp, D., Roos, P., Hanstein, W. G., Chabot, G. G., and Nef, P. (1997) *Arch. Biochem. Biophys.* **337,** 62–68.
17. Strotkamp, D., Roos, P. H., and Hanstein, W. G. (1995) *Biochim. Biophys. Acta* **1260,** 341–344.
18. Nagata, K., Murayama, N., Miyata, M., Shimada, M., Urahashi, A., Yamazoe, Y., and Kato, R. (1996) *Pharmacogenetics* **6,** 103–111.
19. Kirita, S., and Matsubara, T. (1993) *Arch. Biochem. Biophys.* **307,** 253–258.
20. Komori, M., and Oda, Y. (1994) *J. Biochem.* **116,** 114–120.
21. Miyata, M., Nagata, K., Shimada, M., Yamazoe, Y., and Kato, R. (1994) *Arch. Biochem. Biophys.* **314,** 351–359.
22. Kolars, J. C., Lown, K. S., Schmiedlin-Ren, P., Gosh, M., Fang, C., Wrighton, S. A., Merion, R. M., and Watkins, P. B. (1994) *Pharmacogenetics* **4,** 247–259.
23. Mahnke, A., Roos, P. H., Hanstein, W. G., and Chabot, G. G. (1996) *Biochem. Pharmacol.* **51,** 1579–1582.
24. Schuetz, E. G., Wrighton, S. A., Barwick, J. L., and Guzelian, P. S. (1984) *J. Biol. Chem.* **259,** 1999–2006.
25. Huss, J. M., Wang, S. I., Astrom, A., McQuiddy, P., and Kasper, C. S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 4666–4670.
26. Chang, T. K. H., Teixeira, J., Gil, G., and Waxman, D. J. (1993) *Biochem. J.* **291,** 429–434.
27. Pampori, N. A., and Shapiro, B. H. (1996) *Molecular Pharmacology* **50,** 1148–1156.
28. Chen, J., Murray, M., Liddle, C., Jiang, X-M., and Farrell, (1995) *Hepatology* **22,** 580–587.
29. Farrell, G. C., Koltai, A., Zaluzny, Z., and Murray, M. (1986) *Gastroenterology* **90,** 299–305.
30. Frohman, M. A., Dush, M. K., and Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8998–9002.
31. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
32. Yanagimoto, T., Itoh, S., Sawada, M., Hashimoto, H., and Kamataki, T. (1994) *Biochim. Biophys. Acta* **1201,** 405–410.
33. Sundseth, S. S., Alberta, J. A., and Waxman, D. J. (1992) *J. Biol. Chem.* **267,** 3907–3914.
34. Legraverend, C., Mode, A., Westin, S., Strom, A., Eguchi, H., Zaphiropoulos, P. G., and Gustafsson, J.-A. (1992) *Mol. Endocrinol.* **6,** 259–266.
35. Westin, S., Strom, A., Gustafsson, J.-A., and Zaphiropoulos, P. G. (1990) *Mol. Pharmacol.* **38,** 192–197.
36. Waxman, D. J., Pampori, N. A., Ram, P. A., Agrwal, A. K., and Shapiro, B. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6868–6872.
37. Subramanian, A., Teixeira, J., Wang, J., and Gil, G. (1995) *Mol. Cell. Biol.* **15,** 4672–4682.