*Biochemical Pharmacology*, Vol. 33, No. 3, pp. 509–512, 1984.
Printed in Great Britain.

0006–2952/84 $3.00 + 0.00


# PRELIMINARY COMMUNICATION

## POSTNATAL DEVELOPMENT OF CONSTITUTIVE FORMS OF CYTOCHROME P-450 IN LIVER MICROSOMES OF MALE AND FEMALE RATS

Kaori Maeda, Tetsuya Kamataki*+, Tsuneji Nagai, and Ryuichi Kato*

Department of Pharmaceutics, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142 and *Department of Pharmacology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan

*(Received 12 December 1983; accepted 15 December 1983)*

In a previous paper (1), we reported on the purification of constitutive forms of cytochrome P-450, namely P-450-male and P-450-female, from liver microsomes of male and female rats, respectively. Immunochemical examinations of these hemoproteins showed that P-450-male and P-450-female were detectable specifically in respective liver microsomes of adult male and female rats. The synthesis of P-450-male was apparently dependent on testosterone, and that of P-450-female was dependent on estradiol.

Thus, in this study we examined the postnatal development of P-450-male and P-450-female. We show herein that P-450-female is primarily synthesized before the occurrence of P-450-male in male rats. This and other results support the view that the synthesis of unknown pre-existing forms of cytochrome P-450 is depressed in association with the appearance of P-450-male and P-450-female during postnatal periods before sexual maturation.

### MATERIALS AND METHOD

Animals Male and female rats of Sprague-Dawley strain were used throughout this study.

Purification of P-450-male and P-450-female Liver microsomes from untreated male and female rats (8-10 weeks old) were solubilized with cholate. P-450-male and P-450-female were purified from the cholate-solubilized microsomes as described previously (1,2). The specific contents of P-450-male and P-450-female were 11.7 and 13.2 nmol/mg protein, respectively.

Assay methods An incubation mixture for the assay of the N-demethylation of aminopyrine and the hydroxylation of benzo(a)pyrene consisted of liver microsomes (approximately 1 and 0.25 mg protein, respectively), an NADPH-generating system (6 mM magnesium chloride, 8 mM glucose 6-phosphate, 1 unit/ml of incubation mixture of glucose 6-phosphate dehydrogenase and 0.8 mM NADP), 0.1 M sodium potassium phosphate (pH 7.4), 0.1 mM EDTA and the substrate (5 mM aminopyrine and 0.1 mM benzo(a)pyrene) in a final volume of 1.0 ml. Aminopyrine

+ To whom all correspondence and requests for reprints should be addressed.

N-demethylase activity was estimated by determination of formaldehyde by the method of Nash (3). Benzo(a)pyrene hydroxylase activity was estimated fluorometrically as described by Nebert and Gelboin (4). 3-Hydroxybenzo(a)pyrene was used as the standard. Incubations were started by addition of NADPH-generating system and carried out at 37° for 15min, aerobically. Radial immunodiffusion assay of liver microsomes for the content in liver microsomes of P-450-male and P-450-female was conducted by the method reported by Thomas et al (5). Immunoglobulin G previously purified by immunoadsorption (1) was impregnated in agarose gels at a concentration of 0.5 mg/ml. Liver microsomes were solubilized with cholate and Emulgen 911 (Kao Atlas, Japan) at a concentration of 2 mg/ml (1). The content of cytochromes P-450 and $b_5$ were determined according to the methods of Omura and Sato (6) and Omura and Takesue (7), respectively. Protein was determined by the method of Lowry et al (8).

## RESULTS AND DISCUSSION

Postnatal development of P-450-male and P-450-female is shown in Figs. 1 and 2. P-450-male and P-450-female were not detectable until 14 days of age.

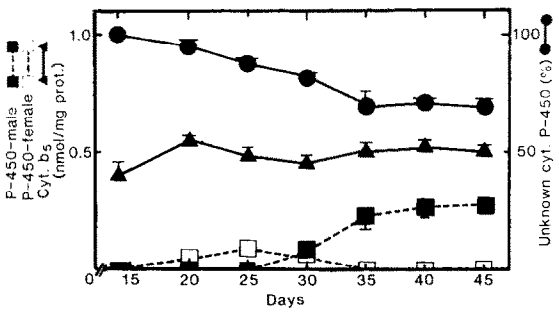


Fig. 1 Postnatal development of P-450-male and P-450-female in liver microsomes of male rats. Vertical bars represent the standard deviation of results (n=4 except for 45 days old, at which n=3).

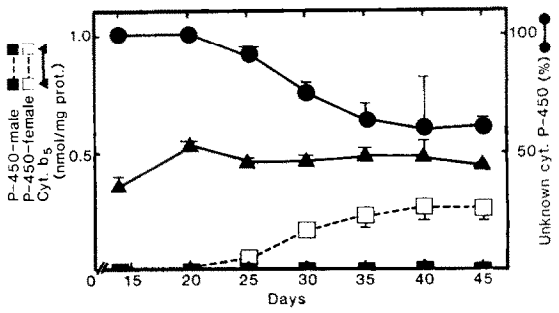


Fig. 2 Postnatal development of P-450-male and P-450-female in liver microsomes of female rats. Vertical bars represent the standard deviation of results (n=4 except for 45 days old, at which n=3).

As can be seen, P-450-female appeared in liver microsomes of female rats 25 days after birth: it increased with age until 40 days. P-450-female was also detectable in liver microsomes of male rats between 20 and 30 days after birth. In association with the decrease in the amount of P-450-female, P-450-male appeared in liver microsomes of male rats. The amount of P-450-male reached the plateau level at around 40 to 45 days old. The total cytochrome P-450 measured by the carbon monoxide binding spectra with the same microsomes did not change markedly between 14 and 45 days old in both male and female rats. Therefore, the amount of unknown cytochrome P-450 decreased with the appearance of P-450-male and P-450-female. Similar change in the population of cytochrome P-450 forms in liver microsomes has been reported by Guengerich et al. (9) in the literature, in which they demonstrated that synthesis of forms of cytochrome P-450 are depressed when other forms of cytochrome P-450

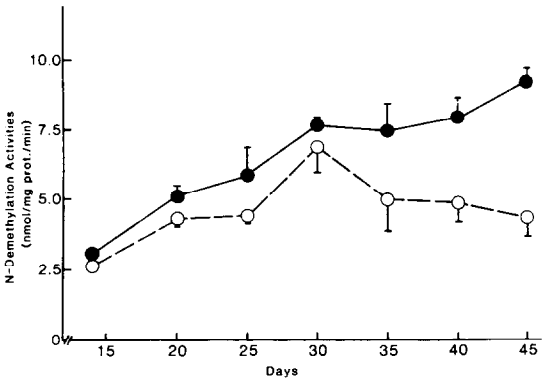


Fig. 3 Change in aminopyrine N-demethylase activity in liver microsomes at the beginning of sexual matulation (n=4 except for 45 days old, at which n=3). (●——●), male rats; (o--o), female rats.

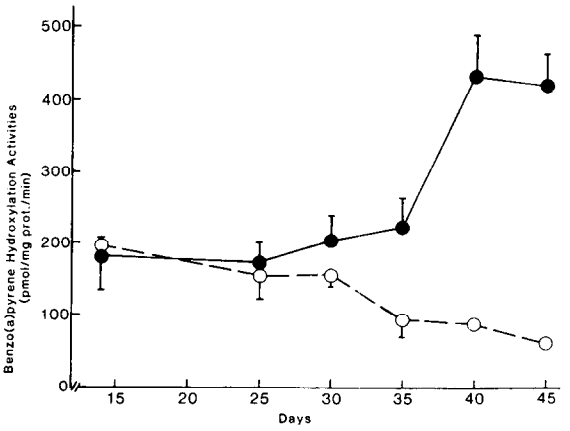


Fig. 4 Change in benzo(a)pyrene hydroxylase activity in liver microsomes at the beginning of sexual matulation (n=4 except for 45 days old, at which n=3). (●——●), male rats; (o--o), female rats.

are induced by β-naphthoflavone, phenobarbital and polychlorinated biphenyl (PCB). Our results indicate that the synthesis of pre-existing forms of cytochrome P-450 is also depressed without exogenous inducers in association with the beginning of sexual maturation. Conney et al. (10) reported that testosterone 6β-, 7α- and 16α-hydroxylases are independently decreased or increased with age. The results probably suggested the substitution of cytochrome P-450 forms in postnatal periods. Thus, our results seem to be in good agreement with their results.

Postnatal development of the activity of aminopyrine N-demethylase in liver microsomes is shown in Fig. 3. A moderate amount of aminopyrine N-demethylase activity was observed 2 weeks after birth in male and female rats. The activity gradually increased in the postnatal period. There were no marked differences between male and female rats until 30 days of age. The activity in both males and females increased to a greater extent between 25 and 30 days of age. After 30 days of age, the activity in male rats increased, but that in female rats decreased, resulting in the appearance of a sex difference.

It has been generally accepted that no marked sex-related differences are seen in 3-methylcholanthrene-inducible drug metabolizing enzymes with some exceptions (11). Wiebel and Gelboin (12) demonstrated a significant sex difference in benzo(a)pyrene hydroxylase, which is inducible by 3-methylcholanthrene. The age-dependent sex difference in benzo(a)pyrene hydroxylase activity is shown in Fig. 4. The activities seen in both male and female rats were similar until 25 days of age. The activity in females decreased, while it increased in males. A remarkable increase in the activity of benzo(a)pyrene hydroxylase was seen between 35 and 40 days old in male rats, whereas only a slight increase was observed in aminopyrine N-demethylase. These results lend support to the idea that sex-related differences in aminopyrine N-demethylation and benzo(a)pyrene hydroxylation are caused at least in part by different molecular forms of cytochrome P-450.

It can be confirmed that age-related development of neither benzo(a)pyrene hydroxylation nor aminopyrine N-demethylation activities can be explicable solely by P-450-male or P-450-female, although a portion of aminopyrine N-demethylation activity in male rats after 30 days of age seems to be due to P-450-male, if the activity is subtracted by the basal activity shown as that before 30 days of age.

In conclusion, we propose that the population of cytochrome P-450 forms, even after three or four weeks old, are changed with age, and that multiple forms of cytochrome P-450 in addition to P-450-male and P-450-female are responsible for the occurrence of a sex-related difference of drug metabolism in the rat in accordance with our previous report (13).

ACKNOWLEDGEMENT

This research was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Kamataki, T., Maeda, K., Yamazoe, Y., Nagai, T., and Kato, R. (1983) Arch. Biochem. Biophys., 225, 758-770
2. Kamataki, T., Maeda, K., Yamazoe, Y., Nagai, T., and Kato, R. (1981) Biochem. Biophys. Res. Commun., 103, 1-7
3. Nash, T. (1953) Biochem. J., 55, 416-421
4. Nebert, D. W., and Gelboin, H. V. (1968) J. Biol. Chem., 243, 6242-6249
5. Thomas, P. E., Korzeniowski, D., Ryan, D., and Levin, W. (1979) Arch. Biochem. Biophys., 192, 524-532
6. Omura, T., and Sato, R. (1964) J. Biol. Chem., 239, 2379-2385
7. Omura, T., and Takesue, S. (1970) J. Biochem., 67, 249-257
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem., 193, 265-275
9. Guengerich, F. P., Dannan, G. A., Wright, S. T., Martin, M. V., and Kaminsky, L. S. (1982) Biochemistry, 21, 6019-6030
10. Conney, A. H., Levin, W., Jacobson, M., Kuntzman, R., Cooper, D. Y., and Rosenthal, O. (1969) In: Microsomes and Drug Oxidations, (J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, and G. R. Mannering, ed.), pp 279-295, New York, Academic Press
11. Kato, R. (1974) Drug Metab. Rev., 3, 1-32
12. Wiebel, F. J. and Gelboin, H. V. (1975) Biochem. Pharmacol., 24, 1511-1515
13. Kamataki, T., Maeda, K., Yamazoe, Y., Nagai, T., and Kato, R. (1982) Life Sci., 31, 2603-2610