ORGAN SPECIFICITY OF THE SEX DEPENDENT REGULATION OF ARYL HYDROCARBON HYDROXYLASE (AHH) IN RAT

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SUMMARY:

Aryl hydrocarbon hydroxylase (AHH) activity was measured in liver, kidneys and lungs of control and gonadectomized Sprague-Dawley rats. There was a six-fold difference in hepatic AHH activity between male and female rat. Activity in male was highest, castration reduced this activity by about 50%, whereas ovariectomy had little effect on activity in female. No sex difference in lung AHH activity was discernable; on the other hand kidney AHH activity was higher in females than in males. These data suggest that, at least in the rat, sex dependent regulation of microsomal mixed function oxygenases is organ specific.

INTRODUCTION:

Microsomal mixed function oxygenase (MFO) is an enzyme complex which is located in the endoplasmic reticulum of the cell and is involved in the metabolism of drugs, carcinogens and several other xenobiotics (1,2). Aryl hydrocarbon hydroxylase (AHH), one of the MFO enzymes, catalyzes the metabolism of polycyclic aromatic hydrocarbons (1-3). Conventional AHH assay based on the fluorescence of phenolic products of benzo(a)pyrene (BP), especially 3-hydroxy BP[3(oH)BP]and to some extent 9-hydroxy-BP(4), is a very sensitive assay that has served as an indicator of hydrocarbon metabolism in several human and animal tissues (5-7). In studies on several inbred strains of mice, inducibility of hepatic AHH activity was positively correlated with the formation of reactive BP-metabolites that bind to proteins (8) and to DNA (9), as tested in in vitro incubation systems. These reactive metabolites are believed to be epoxides (10) and are implicated in carcinogenesis and mutagenesis (11), either by themselves or subsequently due to further metabolism (12). Several factors, including age, sex and nutritional status, modify various MFO activities (1, 13,14) and recently, while our studies were in progress, these factors have also been reported to affect hepatic AHH activity (15,16). In this paper we compare the effects of sex and gonadectomy on AHH activity in liver, kidney and lung.

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MATERIALS AND METHODS :

Chemicals :

Benzo(a)pyrene was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin and it was purified by recrystallization from benzene several times. Spectral grade acetone and hexane were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin and J. T. Baker Chemical Company, Phillipsburg, New Jersey, respectively. NADP, DL-isocitrate and isocitrate dehydrogenase were obtained from Sigma Chemical Co., St. Louis, Missouri. Fluorescence measurements were made with an Aminco-Bowman spectrofluorometer. 3-OH-BP was used as a reference standard for the fluorescence determinations and was kindly provided by Dr. Harry Gelboin of the National Cancer Institute, Bethesda, Maryland.

Gonadectomy :

Sixty days old male and female Sprague-Dawley rats were either sham operated or gonadectomized via a ventral abdominal incision. Following surgery all animals were caged individually and allowed to recover for one week; at the termination of this period two rats from each treatment group were caged together and used 7 days later.

Preparation of Microsomes:

All animals received food and water ad libitum till the day of sacrifice, and were maintained under identical conditions. Animals were decapitated, liver excised, perfused with cold isotonic salt solution and homogenized in ice cold sucrose (0.25M) solution containing 1 mM ethylenediamine tetracetic acid disodium salt (EDTA). The homogenate was centrifuged at 15,000 x g for 15 min. and the resulting supernatant collected and centrifuged at 105,000 x g for 90 min. to obtain the microsomal pellet which was suspended in 0.25 M sucrose. All operations were carried out at 4^{o}.

For the isolation of kidney microsomes, kidneys from each rat were pooled separately, chopped up with scissors and rinsed several times with sucrose-EDTA solution and then treated, as described above for liver, for the isolation of microsomes. In the case of lung tissue, lungs from each animal were pooled, chopped up with scissors, rinsed several times with 0.25 M sucrose and then homogenized in the same solution. The homogenate was centrifuged at 15,000 x g for 15 min and the resulting supernatant was directly used for AHH assay. Protein in various subcellular preparations was determined by the method of Lowry et al (17).

Measurement of AHH activity

AHH activity was measured with liver and kidney microsomes and with lung 15,000 x g supernatant by a modification of the method reported by Nebert and Gelboin (18). Modifications introduced involved the use of the micromethod recently reported

for the measurement of AHH activity in cultured human lymphocytes (19).

The reaction was carried out in 1 ml of the incubation mixture using 0.1 mM BP and 50ug of hepatic microsomal protein or 400 ug of kidney microsomal protein or 100 ul of 15,000 x g supernatant of lung homogenate (0.2 gm/ml). The composition of the incubation mixture was as follows: Tris (0.05M); $MgCl_2$ (3m M); sucrose (0.2 M) buffer pH, 7.6; $MnCl_2$, 1m M; and an NADPH-generating system consisting of NADP (0.33 mM), DL-isocitrate (8m M), and Sigma type IV isocitrate dehydrogenase (10ug protein/ml). The incubation mixture without the enzyme and BP was prewarmed at 37° for 15 min to generate an adequate amount of NADPH needed for the reaction. After preincubation, enzyme source in 100ul aliquot was added, followed immediately by the addition of BP to start the reaction. Incubation was carried out at 37° for 15 min, during which time the reaction rate was linear, and then 4 ml of acetone: hexane (1:3) solvent was added and mixture extracted. 3 ml of the organic phase was transferred to a conical centrifuge tube and extracted with 0.5 ml of 1N NaOH. Organic phase was aspirated and the phenolic metabolites in alkali phase were quantitated by the measurement of their fluorescence, using 396 nm wavelength for excitation and 522 nm wavelength for emission. Fluorescence was compared with a standard curve obtained with 3-OH-BP. AHH activity is expressed as p mole equivalent of 3-OH-BP produced during 15 min. incubation by 1 mg of microsomal protein or 15,000 x g supernatant protein. The results reported in this paper have been corrected for zero-time blanks which were less than 0.2% of the control liver activity. Fluorometer was calibrated with a quinine sulfate standard before and during each run.

RESULTS AND DISCUSSIONS

Hepatic AHH activity is reported in Table 1. The activity in the microsomes from female rat , based on protein content, was 17% of that found in the male rat. Castration of the male lowered the activity to 46% of the control, which was still 2.7-fold greater than that found in the female. Ovariectomy did not produce any discernable effect on the activity in females. When the activity was related to cytochrome P450 content (Table 1), activity in females increased from 17% to 26% of the activity in males. Similar increase in relative activity was also seen in gonadectomized rats and was due to a slightly lower content of cytochrome P450 in castrated male, control female and ovariectomized female. The relative activity related to cytochrome P450 in male, castrated, female and ovariectomized rats was, respectively, 100, 58, 26 and 22%, compared to 100, 46, 17 and 17%, respectively, when related to microsomal protein. These results are in accord with the reported sex difference in other MFO activities, notably, demethylation of ethylmorphine and 3-methyl-4-methylaminoazobenzene (20), N-demethylation of morphine and aliphatic hydroxylation of hexobarbital and pentobarbital (21). Masry et al (14) also reported that significant differences between male and female rats were seen in some other MFO

TABLE 1

AHH activity of hepatic microsomes from control and gonadectomized male and female rats.

Sex and Treatment	n moles cyt. P450[a] per mg mcs. protein Mean ± S.E.	Activity/mg[a,b] mcs protein Mean ± S.E.	Mean Activity as % Control	Activity/n[a,b] moles Cyt. P450 Mean ± S.E.	Mean activity as % Control
Male	1.09 ± 0.06(4)[c,e]	6240 ± 932(4)	100	5651 + 569 (4)	100
Castrated	0.88 ± 0.02(4)[d]	2896 ± 263(4)[d]	46	3267 ± 282 (4)[d]	58
Female	0.74 ± 0.03(4)[d]	1077 ± 196(4)[d]	17	1432 ± 323 (4)[d]	26
Ovariectomized	0.84 ± 0.03 (4)[d]	1030 ± 154(4)[d]	17	1234 ± 185 (4)[d]	22

a) Abbreviations: cyt., Cytochrome; mcs, microsomal

b) AHH activity was measured as described in the text. Activity expressed as pmole equivalents of 3-hydroxy benzo(a)pyrene formed/15 min.

c) Number of animals is given in the ( ).

d) Significantly different from the male at $P < 0.05$.

e) Cytochrome P450 was determined by the reported method (24), using a mM extinction coefficient of 91.

TABLE 2

AHH activity of kidney microsomes and lung 15,000 x g supernatent from control and gonadectomized male and female rats.

Sex and Treatment	Kidney Activity/mg mcs[a] protein[a,b] Mean ± S.E.	Mean Activity as % Control	Lung Activity/mg protein in 15,000 x g supernatent Mean ± S.E.	Mean Activity as % Control
Male	207 ± 20(4)[c,d]	100	40 ± 2.4 (4)	100
Castrated	263 ± 36(4)	127	---	---
Female	309 ± 27 (4)	149	36 ± 5.5 (4)	90
Ovariectomized	249 ± 29 (4)	120	---	---

a) Microsomal

b) AHH activity was measured as described in the text. Activity expressed as p mole equivalents of 3-hydroxy benzo(a)pyrene formed/15 min.

c) Number of Animals given in the ( ).

d) Significantly different from female rat at $P \leq 0.05$

activities even after taking into account the differences in contents of cytochrome P450. Thus, data of Masry et al and our own data presented here suggest that sex of the rat causes a quantitative as well as a qualitative difference in MFO. Sex difference in rat is common to several MFO activities. Exceptions noted are hydroxylation of aniline (14,21) and zoxazolamine (1,13,21). While aniline, based on its spectral interaction with hepatic microsomes, belongs to type II class of drugs (22,23) it is difficult to understand why zoxasolamine hydroxylation, which shows similar induction and inhibition specificities as AHH, should behave differently with respect to the sex difference in rat. We have re-examined this question and our recent preliminary studies show a similar pattern of sex difference in zoxazolamine hydroxylase activity as seen with AHH and aminopyrine demethylation (16,21). Therefore, the apparent discrepancy between the effect of sex on AHH, as reported here and by others (15, 16), and on zoxyzolamine hydroxylation reported by Kato and Gillette (13,21) is difficult to explain at present.

In table 2 AHH activity of kidney microsomes and that of lung 15,000 x g supernatant are reported. Kidney microsomes from male had lower activities than those from other groups of rats: no sex difference in lung AHH activity was discernable. These data are very striking when compared with liver AHH activity which shows a profound sex-related difference favouring male, namely male rat has 6-fold greater AHH activity in the liver than female. Furthermore, these results also suggest that, at least in rats, regulation of MFO, as influenced by sex, is organ dependent. By implication, some of the organ and sex specific differences in toxicity and carcinogenicity of various xenobiotics in the rat might be explained by findings such as those reported in the present paper.

REFERENCES:

1. Conney, A. H., Pharmacol. Rev. 19, 317 (1967).
2. Daly, J. W., Jerina, D. M. and Witkop, B., Experientia 28, 1129, (1972).
3. Kuntzman, R., Annual Rev. Pharmacol. 9, 21-36, (1969).
4. Yang, S. K., Selkirk, J. K., Plotkin, E. V. and Gelboin, H. V., Cancer Res. 35, 3642, (1975).
5. Busbee, D. L., Shaw, C. R. and Cantrell, E. T., Science 178, 315, (1972).
6. Bast, R. C., Jr., Whitlock, J. P., Jr., Miller, H., Rapp, H. J. and Gelboin, H. V., Nature 250, 664, (1974).
7. Gelboin, H. V., Kinoshita, N. and Wiebel, F., Federation Proc. 31, 1298, (1972).
8. Nebert, D. W., Robinson, J. R., Niwa, A., Kumaki, K. and Poland, A. P., J. Cell Physiol. 85, 393, (1975).
9. Gurtoo, H. L. and Sebring, C., Fed. Proc. 34, 755, (1975).
10. Grover, P. L., Hewer, A. and Sims, P., FEBS Letters 18, 76, (1971).

11. Ames, B. N., Durston, W. E., Yamasaki, E. and Lee, F. D., Proc. Natl. Acad. Sci. U.S.A. 70, 2281, (1973).

12. Sims, P., Grover, P. L., Swaisland, A., Pal, K. and Hewer, A., Nature 225, 326, (1974).

13. Kato, R. and Gillette, J. R., J. Pharmacol. Exp. Therap. 150, 285, (1965).

14. El-Masry, S. D., Cohen, G. M. and Mannering, G. J., Drug Metabol. and Dispos. 2, 267, (1974).

15. Lucier, G. W., McDaniel, O. S., Hook, G. E. R., Fowler, B. A., Sonawane, B. R. and Faeder, E., Environ. Health Perspectives September, 199, (1973).

16. Wiebel, F. J. and Gelboin, H. V., Biochem. Pharmacol. 24, 1511, (1975).

17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem. 193, 265, (1951).

18. Nebert, D. W. and Gelboin, H. V., J. Biol. Chem. 243, 6242, (1968).

19. Gurtoo, H. L., Bejba, N. and Minowada, J., Cancer Res. 35, 1235, (1975).

20. El-Masry, S. D. and Mannering, G. J., Drug Metabol. and Dispos. 2, 279, (1974).

21. Kato, R. and Gillette, J. R., J. Pharmacol. and Expt. Therap. 150, 279, (1965).

22. Schenkman, J. B., Remmer, H. and Estabrooks, R. W., Mol. Pharmacol. 3, 113, (1967).

23. Gillette, J. R., Davis, D. C. and Sesame, H. A., Annu. Rev. Pharmacol. 12, 57, (1972).

24. Mazel, P. In: "Fundamentals of Drug Metabolism and Drug Disposition", (Eds., LaDu, B. N., Mandell, H. G. and Way, E. L.) pp. 546-582, The Williams and Wilkins Co., Baltimore, Md., 1972.