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EPOXIDE-METABOLIZING ENZYME ACTIVITIES AND CYTOCHROME P-450 CONTENT OF RAT OVARIES DURING PREGNANCY

Hasan Mukhtar, Richard M. Philpot, and John R. Bend

Laboratory of Pharmacology, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709

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SUMMARY:

Glutathione S-transferase activity was determined in 176,000g supernatant fraction of ovaries, adrenals, and liver and in the serum of virgin, pregnant, lactating, and post-lactating female rats. A 90% increase in glutathione S-transferase specific activity was observed in the ovaries of pregnant rats whereas activity in the other tissues was not affected by pregnancy. The increase in ovarian glutathione S-transferase activity was not a function of the estrous cycle of the rat. The activity reached a maximum in midpregnancy and then remained constant until late pregnancy. The specific content of cytochromes P-450 and b_5 in microsomes prepared from rat ovaries was induced 3.7- to 4.7-fold during mid- and late pregnancy. No concomitant changes occurred in mitochondrial cytochrome P-450 concentrations. Microsomal aryl hydrocarbon hydroxylase and epoxide hydrase activities of ovaries from pregnant rats were not affected.

INTRODUCTION:

The epoxides formed by microsomal mixed-function oxidation of several olefinic and polycyclic aromatic hydrocarbons (1-4) are implicated as at least one of the chemical species responsible for the toxic effects of the parent hydrocarbon (5,6). These highly reactive electrophiles are biotransformed by hydration to the corresponding diols or trans-dihydrodiols by microsomal epoxide hydrase (3,7,8) and by conjugation with glutathione, a reaction catalyzed by a family of soluble enzymes, the glutathione S-transferases (9,10). These two enzymatic pathways, at least for alkene oxides, are detoxification reactions. However, in the case of benzo(a)pyrene, the conversion of benzo(a)pyrene 7,8-

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oxide to benzo(a)pyrene 7,8-dihydrodiol by epoxide hydrase can be thought of as an activation pathway, since the diol formed is the precursor for the isomeric benzo(a)pyrene 7,8-dihydrodiol-9,10 epoxides which are potent mutagens and carcinogens (11, 12). Similar activation reactions occur with some other polycyclic aromatic hydrocarbons. Recently, we have studied the development of epoxide-metabolizing enzyme activities in steroidogenic tissues of the rat (13). Relatively high (compared to liver) enzyme activities were present in ovaries which may protect against the toxic effects of circulating electrophiles in this important target organ.

The epoxide-detoxification systems in ovarian tissue may be particularly important for protection of the oocyte. Numerous studies have been conducted on the effects of pregnancy and/or steroids on oxidative metabolism of drugs and other xenobiotics. In both rabbits and rats, pregnancy either has no effect or inhibits the hepatic mixed-function oxidase system (14, 15). Neale and Parke (14) reported that during late pregnancy in rat there is a 40% increase in liver weight and a 25% decrease in cytochrome P-450 concentration per gram of liver; no such changes occurred during mid-pregnancy. On the other hand, considerable evidence has also been accumulated which suggests that the pulmonary mixed-function oxidase activities toward benzphetamine and N,N-dimethylaniline as well as cytochrome P-450 concentrations are almost doubled in pregnant versus nonpregnant rabbits (16).

To our knowledge neither epoxide-metabolizing enzyme activities nor mixed-function oxidation of polycyclic hydrocarbons have been previously studied in ovaries of pregnant rats. In this communication we report that the concentrations of ovarian microsomal cytochromes P-450 and b_5 and the soluble fraction glutathione S-transferase activities toward SO, 4,5-BPO, DNCB, and DCNB increase significantly during pregnancy.

Abbreviations used: SO, styrene 7,8-oxide; 4,5-BPO, benzo(a)pyrene 4,5-oxide; DNCB, 2,4-dinitro-1-chlorobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; AHH, aryl hydrocarbon hydroxylase; HEPES, N-2-hydroxylpiperazine-N'-2-ethane sulfonic acid.

MATERIALS AND METHODS:

Styrene oxide-8-^{14}C (sp. act., 0.497 mCi/mmol, radiochemical purity > 98.5%) was purchased from New England Nuclear, Boston, Mass. Benzo(a)pyrene 4,5-oxide-G-^{3}H (sp. act., 10 mCi/mmol, radiochemical purity > 99%) and unlabeled 4,5-BPO were purchased from Midwest Research Institute, Kansas City, Mo. Unlabeled styrene oxide was a product of Eastman Organic Chemicals, Rochester, N. Y. HEPES , benzo(a)pyrene, glucose 6-phosphate, glucose 6-phosphate dehydrogenase (type XI), NADP, and glutathione were procured from Sigma Chemical Co., St. Louis, Mo. DCNB and DNCB were supplied by Eastman Organic Chemicals and the Aldrich Chemical, Milwaukee, Wis., respectively, and were recrystallized from ethanol prior to use.

Preparation of mitochondrial, microsomal, and microsomal supernatant fractions from ovaries, liver, and adrenals: Charles River female Sprague-Dawley rats were used in these studies. The day the sperm plug was observed in the vagina was taken as the beginning of pregnancy. At specified days after the onset of pregnancy, rats were sacrificed by decapitation, and the ovaries, adrenals, and livers were surgically removed. The organs were placed on ice and the fatty and connective tissues were removed. Tissues from 8-10 animals were pooled, cut up with scissors, and homogenized in 10 volumes of 0.15 M KCl-0.02 M HEPES, pH 7.4, using 8 passes in a glass homogenizer fitted with a motor driven teflon pestle. The homogenate was centrifuged at 600*g* for 10 min to precipitate nuclei, undisrupted cells, and cell debris. The resultant pellet was resuspended and centrifuged again at 600x*g* for 10 min. The 600x*g* supernatant fractions were pooled and centrifuged at 8,000x*g* for 15 min to sediment mitochondria. The mitochondrial pellet was resuspended, centrifuged at 8,000x*g* for 15 min, and the supernatant fractions were combined. The pellet was washed once more in buffer and the washed mitochondria were suspended in 2 ml of ice-cold 0.25 M sucrose. The pooled 8,000x*g* supernatant fractions were centrifuged at 176,000x*g* for 45 min using a 60 Ti rotor in a Beckman L 3-50 ultracentrifuge. The supernatant was carefully aspirated to another tube and was used as the enzyme source for glutathione *S*-transferase activity. The microsomal pellet was resuspended in KCl-HEPES and was recentrifuged at 176,000x*g* for 20 min. The pellet was suspended in 0.25 M sucrose and was used for microsomal spectral analysis and enzyme assays.

Enzyme Assays: Microsomal epoxide hydrase activity and soluble fraction glutathione *S*-transferase activity with SO or 4,5-BPO as substrates were assayed as described earlier (7). Glutathione *S*-transferase activity with DNCB or DCNB was assayed spectrophotometrically as described by Habig *et al.* (17) using a Unicam SP 1800 dual beam spectrophotometer. A unit of enzyme activity is defined as the amount of enzyme catalyzing the formation of 1 nmol glutathione conjugate per min. Specific activity is expressed as units of enzyme activity per mg protein. AHH activity was determined by the method of Dehnen *et al.* (18). The conditions employed were those reported by Pohl *et al.* (19). Protein content was determined according to the procedure of Lowry *et al.* (20) using bovine serum albumin as the reference standard.

Spectral Analysis: Cytochrome P-450 and b_5 concentrations were determined by the methods of Omura and Sato (21).

RESULTS:

Glutathione *S*-transferase activity was determined in liver, adrenals, ovaries, and serum of virgin, 19- to 20-day pregnant, lactating, and 10- and 30-day post-lactating female rats. These data are summarized in Table 1. Ovarian glutathione *S*-transferase activity increased almost 2-fold in late

TABLE 1

GLUTATHIONE S-TRANSFERASE ACTIVITY IN SERUM, ADRENALS, LIVER, AND OVARIES OF FEMALE VIRGIN, PREGNANT, LACTATING, AND POST-LACTATING RATS WITH STYRENE 7,8-OXIDE AS SUBSTRATE

	LIVER	ADRENALS	OVARIES	SERUM	nmoles conjugate formed/min/ml serum
Virgin	161 ± 8[a]	140 ± 5	95 ± 4	0.79 ± 0.03	75.1 ± 4.2
Pregnant (19-20 days)	182 ± 11	138 ± 6	182 ± 14[b]	0.75 ± 0.03	72.0 ± 4.3
Lactating	199 ± 10	142 ± 7	249 ± 23[b]	0.76 ± 0.04	61.3 ± 5.6
10 Day Post-Lactating	174 ± 8	131 ± 6	241 ± 24[b]	0.79 ± 0.05	66.2 ± 5.3
30 Day Post-Lactating	N.D.[c]	N.D.	112 ± 8	N.D.	N.D.

[a]Mean ± S.D. (N = 4), nmoles product formed/min/mg protein.
[b]$P < 0.05$.
[c]N.D. = not determined.

pregnancy as compared to virgin female rats when SO was used as the substrate. The specific activity remained elevated during lactation and returned to near-control levels by 30 days after cessation of lactation. No comparable changes were detected in hepatic, adrenal, or serum glutathione S-transferase specific activities. Consequently, ovarian enzyme activities during pregnancy were selected for more detailed study.

To test if glutathione S-transferase activity in ovaries was influenced by the estrous cycle, enzyme activities were measured during estrus, metestrus, diestrus, and proestrus. As shown in Table 2, no statistically significant changes were observed in specific glutathione S-transferase activities during the various stages of the estrous cycle. Ovarian glutathione S-transferase activity was studied, with SO as substrate, as a function of the time (days) after onset of pregnancy. As shown in Figure 1, a 61% increase in ovarian specific glutathione S-transferase activity was observed 7 days after pregnancy; at 11 days of gestation specific enzyme activity was increased by 78% over nonpregnant controls. Activity then remained relatively constant throughout

TABLE 2

GLUTATHIONE S-TRANSFERASE ACTIVITY IN 176,000xg SUPERNATANT FRACTIONS OF RAT OVARIES DURING DIFFERENT STAGES OF THE ESTROUS CYCLE USING STYRENE 7,8-OXIDE AS SUBSTRATE

CYCLE STAGE	NMOLES CONJUGATE/MIN/MG PROTEIN
Estrus	154 ± 16[a]
Midestrus	124 ± 14
Diesterus	151 ± 10
Proestrus	147 ± 12

[a]Mean ± S.D. (N = 4).

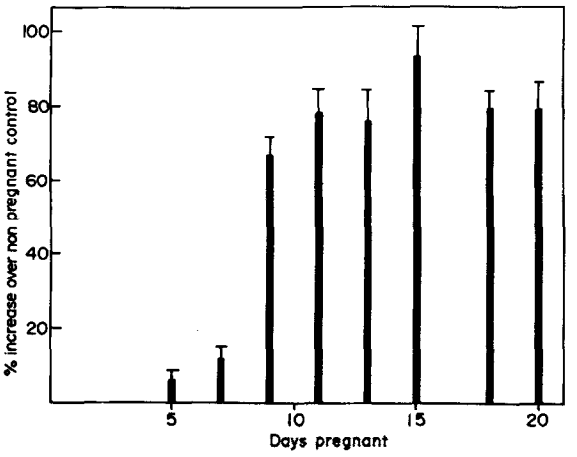

Figure 1. Percent increase in ovarian glutathione S-transferase specific activity, with styrene 7,8-oxide as substrate, as a function of time (days) after onset of pregnancy in female rats. Values are reported as the means of three determinations. Identical non-pregnant controls were run with each experiment. There were no significant variations in these control animals.

the later stages of pregnancy. Glutathione S-transferase activities of control and pregnant (19-20 days) rats with DCNB and DNCB, two substrates widely used for assaying enzyme activity, and with 4,5-BPO, a mutagenic K-region arene

TABLE 3

SPECIFIC GLUTATHIONE S-TRANSFERASE ACTIVITIES IN CONTROL AND PREGNANT RAT OVARIES WITH 1,2-DICHLORO-4-NITROBENZENE, 1-CHLORO-2,4-DINITROBENZENE, STYRENE 7,8-OXIDE, AND BENZO(a)PYRENE 4,5-OXIDE AS SUBSTRATES

	NMOL PRODUCT · MIN^{-1} · MG $PROTEIN^{-1}$		
SUBSTRATE	CONTROL NON PREGNANT	LATE PREGNANCY (19-20 DAYS)	PERCENT INCREASE
Styrene 7,8-Oxide	108 ± 9[a]	229 ± 13[b]	112
Benzo(a)pyrene 4,5-Oxide	12.6 ± 0.9	16.7 ± 1.1[c]	33
1,2-Dichloro-4-Nitrobenzene	1.54 ± 0.11	2.06 ± 0.19[c]	34
1-Chloro-2,4-Dinitrobenzene	64.8 ± 2.2	90.7 ± 3.3[c]	40

[a]Mean ± S.D. (N = 4).

[b]$P < 0.01$.

[c]$P < 0.05$.

oxide, are shown in Table 3. A 112% increase in specific activity was observed with styrene oxide whereas 33, 34 and 40% increases were observed when 4,5-BPO, DCNB, and DNCB, respectively, were employed as substrates.

Microsomal AHH and epoxide hydrase activities (with 4,5-BPO as substrate) and cytochrome P-450 and cytochrome b_5 contents, as well as mitochondrial cytochrome P-450 concentrations, were determined at various stages of pregnancy (Table 4). Significant increases in microsomal cytochromes P-450 and b_5 were observed during pregnancy; the specific microsomal P-450 content was 2-, 3.7-, and 4.7-fold higher in the ovaries of rats during early, mid- and late pregnancy, respectively (relative to nonpregnant control rats). Similar increases in the concentration of microsomal cytochrome b_5 were observed. No statistically significant increase in specific microsomal epoxide hydrase activity was observed during any stage of pregnancy, nor was any increase noted in ovarian

TABLE 4

MICROSOMAL CYTOCHROME P-450 AND b_5 CONTENTS, ARYL HYDROCARBON HYDROXYLASE, EPOXIDE HYDRASE ACTIVITIES AND MITOCHONDRIAL P-450 CONTENT IN RAT OVARIES AT VARIOUS STAGES OF PREGNANCY

PARAMETER	CONTROL NONPREGNANT	EARLY PREGNANCY (4-5 days)	MIDPREGNANCY (11-14 days)	LATE PREGNANCY (19-20 days)
Mitochondrial Cytochrome P-450[a]	0.14 ± 0.04 (5)[b]	0.11, 0.13	0.08, 0.08	0.09 ± 0.01 (5)
Microsomal Cytochrome P-450[a]	0.03 ± 0.01 (5)	0.06, 0.07[e]	0.12, 0.10[f]	0.14 ± 0.01 (5)[f]
Microsomal Cytochrome b_5[a]	0.04 ± 0.01 (5)	0.07, 0.07[e]	0.13, 0.11[f]	0.10 ± 0.01 (3)[f]
AHH Activity[c]	3.16 ± 0.32 (4)	3.51 ± 0.41 (4)	3.76 ± 0.52 (4)	3.27 ± 0.40 (4)
Epoxide Hydrase[d]	0.60 ± 0.47 (3)	0.64 ± 0.41 (3)	0.72 ± 0.32 (3)	0.84 ± 0.42 (3)
Ovary Weight (mg)	49.6 ± 2.4 (6)	53.4 ± 2.6 (6)	62.8 ± 3.2 (6)	71.7 ± 5.1 (6)

[a] nmoles/mg microsomal or mitochondrial protein
[b] mean ± S.D. (N).
[c] pmoles 3-hydroxybenzo(a)pyrene/min/mg protein.
[d] nmoles benzo(a)pyrene 4,5-dihydrodiol formed/min/mg protein.
[e] $P < 0.05$.
[f] $P < 0.01$.

mitochondrial cytochrome P-450 content or in microsomal AHH activity. Data on the corresponding hepatic values are also given in Table 5. In contrast to ovary, slightly lower microsomal AHH and epoxide hydrase specific activities and cytochrome P-450 content were observed in liver during late pregnancy.

DISCUSSION:

Existing epidemiologic data suggest that exposure to environmental pollutants during the first 20 years of life may be important in the etiology of human ovarian cancer (22). Several of these pollutants, e.g., the polycyclic aromatic hydrocarbons, benzo(a)pyrene, 3-methylcholanthrene, and 7,12-dimethylbenzanthracene, have been shown to initiate ovarian granulosa cell tumors in mice (23). These hydrocarbons are apparently not carcinogenic themselves but require metabolic activation by microsomal cytochrome P-450-dependent monooxygenases. The reactive epoxides formed are inactivated by conjugation with glutathione, as well as by other mechanisms. Recently, much attention has focused on the potential involvement of glutathione S-transferases as detoxification

TABLE 5

MICROSOMAL CYTOCHROME P-450 AND b_5 CONTENTS AND ARYL HYDROCARBON HYDROXYLASE AND EPOXIDE HYDRASE ACTIVITIES IN RAT LIVER AT VARIOUS STAGES OF PREGNANCY

PARAMETER	CONTROL NONPREGNANT	EARLY PREGNANCY (4-5 days)	MIDPREGNANCY (11-14 days)	LATE PREGNANCY (19-20 days)
Cytochrome P-450[a]	0.77 ± 0.25 (4)[b]	0.51, 0.51	0.54, 0.53	0.68, 0.85
Cytochrome b_5[a]	0.50, 0.61	N.D.	N.D.	0.50, 0.56
AHH Activity[c]	1.56 ± 0.12 (3)	1.42 ± 0.14 (3)	1.51 ± 0.17 (3)	1.25 ± 0.12 (4)[d]
Epoxide Hydrase Activity[c]	10.5 ± 1.32 (4)	11.2 ± 0.98 (4)	10.6 ± 1.0 (4)	7.9 ± 0.92 (4)[d]
Liver Weight (g)	10.7	12.7	14.8	16.5

[a] nmoles/mg microsomal protein.
[b] Mean ± S.D. (N).
[c] nmoles product/min/mg protein.
[d] $P < 0.01$.
N.D. = Not determined.

enzymes (for review, see 10 and 24) for reactive electrophiles including arene and alkene oxides. The presence of xenobiotic-metabolizing enzyme activities in extrahepatic tissues suggests that these localized enzymes may be a factor in the determination of chemical-mediated target organ toxicity, even though their total activity is very low relative to liver. For example, ovarian glutathione S-transferase activity in the rat is less than 0.2% of that found in the liver.

Available data indicates that there is no increase in hepatic drug-metabolizing enzyme activities during pregnancy; in fact, a decrease has occasionally been observed (14). The ovarian enzymes may play a role in the biotransformation of drugs and xenobiotics that have escaped metabolic conversion by the liver. We found that glutathione S-transferase activities and cytochrome P-450 and b_5 concentrations are significantly increased in ovaries during mid- and late pregnancy. Since the embryo is most susceptible to the effect of some chemical teratogens during the phase of organogenesis, i.e., during mid-pregnancy (25, 26), increased ovarian glutathione S-transferase activities during pregnancy may help to protect the fetus and maternal germ cells from the teratogenic

effects of certain electrophilic xenobiotics. This increase in ovarian glutathione S-transferase activity and in microsomal cytochromes P-450 and b_5 may be under hormonal control. Prolactin and progesterone are known to have profound effects on the development of the corpus luteum during late pregnancy. We are currently investigating the effects of administering various hormones on several ovarian enzymes in nonpregnant rats. The levels of many microsomal steroid metabolizing enzymes such as aromatase, a key enzyme in estrogen biosynthesis, are elevated in mid- and late pregnancy when estrogen biosynthesis is very high (27). Consequently, the observed changes in the levels of cytochrome P-450 and b_5 and in glutathione S-transferase activities during pregnancy may be related to increased steroid biosynthesis.

The increase in ovarian glutathione S-transferase specific activity during late pregnancy was maximal when styrene oxide was employed as substrate. This indicates that there may be a preferential increase in the content of one or more, but not all, ovarian glutathione S-transferases. This is consistent with our observations on partially purified glutathione S-transferases from rabbit liver and lung, where it was found that styrene oxide was not a substrate for all glutathione S-transferases (28). Also the elegant studies of Jakoby and his collegues (17) have earlier demonstrated the presence of seven different glutathione S-transferases in rat liver with overlapping substrate specificities.

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