Drug Induction and Sex Differences of Renal Glutathione S-Transferases in the Rat*

By GIL CLIFTON, NEIL KAPLOWITZ, JOHN D. WALLIN and JOHN KUHNENKAMP
Clinical Investigation Center, Naval Regional Medical Center, Oakland, Calif. 94627, U.S.A.

(Received 29 January 1975)

Treatment of male rats with 3,4-benzopyrene, 3-methylcholanthrene and phenobarbital resulted in the induction of glutathione *S*-aryl- and *S*-aralkyl-transferase activities in kidney cytosol. Benzopyrene produced 77 and 44% increases in aryl and aralkyl activities respectively. Methylcholanthrene caused 73 and 86% increases in the respective activities, whereas phenobarbital treatment increased only aralkyl activity (51%). There was no effect on epoxide or alkyl glutathione *S*-transferase activities with these treatments. Differences were found between the specific activities of the four glutathione *S*-transferases in females and males, with the following female/male ratios: aryl 0.74; aralkyl 2.37; epoxide 1.52; alkyl 1.33. No changes in K_m values were observed relative to drug induction or sex differences. Comparisons are made between the findings of this report and corresponding experiments with liver.

The glutathione *S*-transferases are a group of enzymes thought to be important in drug and carcinogen metabolism (Boyland & Chasseaud, 1969; Pabst *et al.*, 1973). These enzymes function by converting a variety of substrates, e.g. aryl (Booth *et al.*, 1961), epoxide (Boyland & Williams, 1965; Fjellstedt *et al.*, 1973) and alkyl moieties (Johnson, 1966) into glutathione conjugates. Four hepatic glutathione *S*-transferases denoted A, B, C and E, have been purified to homogeneity (Fjellstedt *et al.*, 1973; Habig *et al.*, 1974b; Pabst *et al.*, 1974), and one of these, glutathione *S*-transferase B, has been shown to be identical with ligandin (Habig *et al.*, 1974a), an hepatic cytosolic organic anion-binding protein (Litwack *et al.*, 1971).

Although much attention has been directed at glutathione *S*-transferases in the liver, these activities also have been identified in the kidney. Alkyl- (Boyland & Williams, 1965; Johnson, 1966) and epoxide-transferases (Johnson, 1966) are present in the kidney at specific activities near those found for the same activities in the liver (82 and 45% respectively). However, renal aryl activity exists at approx. 2% of the hepatic activity (Booth *et al.*, 1961; Boyland & Williams, 1965).

The induction of four hepatic glutathione *S*-transferase activities by phenobarbital and polycyclic aromatic hydrocarbons has been described (Kaplowitz *et al.*, 1975). In addition, sex differences have been observed in these hepatic transferase activities (Darby & Grundy, 1972; Kaplowitz *et al.*, 1975).

Therefore it was decided to examine the inducibility and sex differences of these enzymes in the kidney.

Materials and Methods

Animals, treatment and preparation of kidney cytosol

Three groups of ten Sprague-Dawley rats (160–180 g) were given phenobarbital (Sigma Chemical Co., St. Louis, Mo., U.S.A.; 8 mg/100 g, in 0.9% NaCl intraperitoneally daily for 10 days), 3,4-benzopyrene (Sigma Chemical Co.; 1 mg in corn oil intraperitoneally twice daily for 10 days) or 3-methylcholanthrene (Calbiochem, San Diego, Calif., U.S.A.; 1 mg in corn oil intraperitoneally twice daily for 10 days). Two groups of ten controls received either 0.9% NaCl or corn oil. Animals were killed by ether anaesthesia 24 h after the last treatment. The kidneys were perfused *in situ* with 0.01 M-sodium phosphate buffer, pH 7.4, and 20% homogenates (0.01 M-sodium phosphate – 0.25 M-sucrose buffer, pH 7.4) were centrifuged at 105 000g for 60 min in a Beckman L2-65B ultracentrifuge (Beckman Instruments Inc., Fullerton, Calif., U.S.A.). After removal of lipid layers by suction, cytosol fractions were decanted from residue. Samples were divided and stored at –15°C for several months with no resulting loss of activity.

Determination of enzymic activities

Glutathione *S*-aryl-, *S*-aralkyl- and *S*-epoxide-transferase activities were measured kinetically by previously described techniques (Booth *et al.*, 1961;

* Reprint requests to Publications Office, Clinical Investigation Center, Naval Regional Medical Center, Oakland, Calif. 94627, U.S.A.

Pabst *et al.*, 1973) and have been reported in detail (Kaplowitz *et al.*, 1975). Initial reaction rates were measured by observing the formation of GSH conjugates of the various substrates. Assays were done at 37°C in a Beckman DK-2A spectrophotometer. The aryl activity was determined at 344nm with 1.0mM-3,4-dichloronitrobenzene (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.) in 200µl of kidney cytosol; aralkyl activity was measured at 310nm with 0.5mM-*p*-nitrobenzyl chloride (Aldrich Chemical Co.) in 50µl of cytosol; epoxide activity was measured at 360nm with 0.5mM-1,2-epoxy-(*p*-nitrophenoxy)propane in 200µl of cytosol. The aryl activity was measured in 0.1M-sodium phosphate buffer, pH8.0; the aralkyl and epoxide activities were measured in 0.1M-sodium phosphate, pH6.5. In all cases total assay volume was 2.0ml and each assay contained 10mM-GSH (Sigma Chemical Co.) as well as 5% (v/v) ethanol to maintain the solubility of the substrates. Reactions were linear with time and protein concentration.

Glutathione *S*-alkyltransferase activity was measured by using [¹⁴C]methyl iodide (New England Nuclear Corp., Boston, Mass., U.S.A.) in a modification of the assay procedure of Johnson (1966) as described by Kaplowitz *et al.* (1975). Determinations were done in duplicate at 25°C in 66mM-sodium phosphate-2mM-EDTA buffer, pH7.0, with a reaction volume of 3.0ml. Methyl iodide concentration was 1.5mM, and GSH was present at 10mM. Reactions were initiated with 200µl of kidney cytosol. Samples (0.5ml) were withdrawn at 0 and 3min, and each was transferred immediately to a liquid-scintillation counting vial containing 1.0ml of methanol. After drying by gentle heating, the contents of each vial were treated with 1.0ml of hot distilled water followed by 15ml of Aquasol

(New England Nuclear Corp.) before counting for 10min in a Packard Tri-Carb liquid-scintillation spectrometer.

Under the experimental conditions used for the above assays of enzymic activity, spontaneous interaction of substrates was found to be negligible.

Protein concentrations were determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Enzyme specific activities were expressed as nmol of product/min per mg of protein.

Enzymic activities from groups of rats were compared by unpaired Student's *t* test (Batson, 1956).

Results

Induction by polycyclic aromatic hydrocarbons and phenobarbital

Two renal glutathione *S*-transferase activities, aryl and aralkyl, were significantly increased in male rats that had been treated with 3,4-benzopyrene or 3-methylcholanthrene (Table 1). Benzopyrene treatment produced 77 and 44% increases in aryl and aralkyl activities respectively. For animals given methylcholanthrene increases of 73 and 86% (aryl and aralkyl activities respectively) were observed. There was no induction by either drug of the renal epoxide or alkyl activities.

Phenobarbital treatment resulted in a 51% increase in the renal glutathione *S*-aralkyltransferase activity but had no significant effect on the other three activities (Table 1). It should also be noted that there was no difference in enzymic activities between controls treated with saline or corn oil.

Sex differences in enzymic activities

In comparing enzymic activities from kidney cytosol of male and female animals, significant

Table 1. *Drug induction and sex differences of four glutathione S-transferase activities from rat kidney cytosol*

Enzymic activities are expressed as means $\pm$ S.E.M. with units of nmol/min per mg of protein. Each group consists of activities determined in kidney cytosol from ten animals. Results of statistical comparisons of enzymic activities from female versus male animals is given below the value for the male animals and for comparisons of activities from drug-treated males versus control males below the value for the corresponding drug group. N.S., No significant difference in activities between treated and control groups ($P > 0.10$). See the text for details of assay procedures and treatment of animals.

	Glutathione <i>S</i> -transferase activity				
	Control		Phenobarbital-treated	3,4-Benzopyrene-treated	3-Methylcholanthrene-treated
	Female	Male			
Aryl	2.81 $\pm$ 0.12	3.80 $\pm$ 0.29 ($P < 0.005$)	3.80 $\pm$ 0.21 (N.S.)	6.72 $\pm$ 1.32 ($P < 0.005$)	6.58 $\pm$ 0.66 ($P < 0.001$)
Aralkyl	70.0 $\pm$ 4.7	29.5 $\pm$ 2.7 ($P < 0.001$)	44.5 $\pm$ 1.8 ($P < 0.001$)	42.6 $\pm$ 0.33 ($P = 0.005$)	54.9 $\pm$ 0.53 ($P < 0.001$)
Epoxide	12.0 $\pm$ 0.7	7.89 $\pm$ 0.40 ($P < 0.001$)	8.30 $\pm$ 0.47 (N.S.)	8.56 $\pm$ 0.47 (N.S.)	8.35 $\pm$ 0.47 (N.S.)
Alkyl	20.4 $\pm$ 0.8	15.3 $\pm$ 0.8 ($P < 0.001$)	16.3 $\pm$ 1.1 (N.S.)	15.4 $\pm$ 1.1 (N.S.)	16.5 $\pm$ 1.1 (N.S.)

Fig. 1. Kinetics of drug induction and sex differences of renal glutathione *S*-aralkyltransferase activity

Enzymic activities in kidney cytosol from female (■) and male (●) controls, male animals treated with 3,4-benzopyrene (○), 3-methylcholanthrene (△) and phenobarbital (□), were measured at 37°C in 0.1M-sodium phosphate buffer, pH6.5, containing 10mM-GSH and 0.25–0.60mM-*p*-nitrobenzyl chloride.

differences were observed for all the glutathione *S*-transferase activities. Three activities were greater in female rat kidney, i.e. *S*-aralkyl-, *S*-epoxide- and *S*-alkyl-transferases, with female/male activities ratios of 2.37, 1.52 and 1.33 respectively. Aryl activity was higher in cytosol from male kidney with a female/male ratio of 0.74.

Enzyme kinetic studies

The effect of drug induction and sex differences on the kinetics of the four glutathione *S*-transferase activities was determined. A representative Lineweaver-Burk (1934) plot for renal glutathione *S*-aralkyltransferase activity is given in Fig. 1. Kidney cytosol from animals treated with phenobarbital, 3-methylcholanthrene and 3,4-benzopyrene demonstrated an increase in the V_{max} value of GSH-*p*-nitrobenzyl chloride conjugation without a change in the K_m value. In addition, despite the difference in V_{max} values for this activity in male versus female kidney, no difference in K_m values was observed. Similar results with respect to drug induction and sex differences were obtained for the other glutathione *S*-transferase activities in kidney.

Comparison of specific enzyme activities from kidney versus liver

In comparing the enzymic activities for renal transferases with the hepatic activities that have been reported (Kaplowitz *et al.*, 1975), the following kidney/liver ratios of glutathione *S*-transferase activity were calculated: aryl, 0.02; aralkyl, 0.17;

epoxide, 0.68; alkyl, 0.92. The first three of these differences between kidney and liver specific enzyme activities were all highly significant ($P < 0.001$).

Discussion

Induction of renal enzymes, both cytosolic and microsomal, has been reported as follows. Two cytoplasmic enzymes, alcohol dehydrogenase and β -glucuronidase, have been induced in the mouse kidney with the steroid 5 α -androstane-3 α ,17 β -diol (Ohno *et al.*, 1971). Microsomal aryl hydrocarbon hydroxylase is induced in rat kidney after treatment with 3,4-benzopyrene (Grundin *et al.*, 1973) or 3-methylcholanthrene (Gelboin & Blackburn, 1964; Nebert *et al.*, 1972). It is noteworthy, however, that phenobarbital induction of kidney enzymes has not been reported.

The increases in the renal aryl and aralkyl activities after treatment with polycyclic aromatic hydrocarbon were significantly greater than those found for the hepatic activities (Kaplowitz *et al.*, 1975). Induction of the renal aralkyl activity after treatment with phenobarbital was slightly less than that for the hepatic activity. Since the K_m values for the induced renal activities were unaltered by drug treatment (Fig. 1), it is likely that these treatments resulted in increased quantities of the enzymes.

Hepatic glutathione *S*-aryl-, *S*-aralkyl- and *S*-epoxide-transferase activities have been shown to be significantly greater in males compared with female rats, whereas no difference in hepatic glutathione *S*-alkyltransferase has been found (Kaplowitz *et al.*, 1975). In contrast renal aralkyl, epoxide and alkyl activities are greater in females than in males.

The hepatic glutathione *S*-transferases are believed to function as a cytosolic detoxifying system (Boylard & Chasseaud, 1969; Pabst *et al.*, 1973). Kaplowitz *et al.* (1973, 1974) proposed that these enzymes also serve as hepatic cytoplasmic receptors for organic anions which are not substrates for these enzymes. This hypothesis was derived from finding similarities between these enzymes and the organic anion-binding protein, ligandin, and then demonstrating that Indocyanine Green and bilirubin were competitive inhibitors of these activities (Kaplowitz *et al.*, 1973, 1974). Subsequent work by Habig *et al.* (1974a) confirmed these suggestions and demonstrated the identity of ligandin and hepatic glutathione *S*-transferase B. Since the kidney also has glutathione *S*-transferase activities (Kaplowitz *et al.*, 1973) and demonstrable ligandin (Fleischner *et al.*, 1972; Habig *et al.*, 1974a), a similar role may be proposed for these enzymes in the kidney.

The present data suggest at least one striking difference between the transferases in kidney and liver. Although phenobarbital induction of hepatic aralkyl activity has been shown to parallel closely

induction of aryl activity, with respect to both quantitative and sequential induction, renal aralkyl activity is induced selectively by phenobarbital. The significance of this difference in induction between organs is further enhanced by the findings of Habig *et al.* (1974a,b). These workers have purified four hepatic *S*-transferases, A, B, C and E, and have shown that each of the four proteins metabolizes *p*-nitrobenzyl chloride (aralkyl activity) in addition to other substrates such as 3,4-dichloronitrobenzene (aryl), 1,2-epoxy-(*p*-nitrophenoxy)propane (epoxide) and iodomethane (alkyl activity). Therefore in the liver any induction of aryl-, alkyl- or epoxide-transferase activity would be accompanied by increased aralkyl activity.

This study was supported through funds provided by the Bureau of Medicine and Surgery, Navy Department, for CIP 5-48-498C. The opinions and assertions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large.

References

- Batson, H. D. (1956) *An Introduction to Statistics in the Medical Sciences*, Chicago Medical Book Co., Chicago
- Booth, J., Boyland, E. & Sims, P. (1961) *Biochem. J.* **79**, 516-524
- Boyland, E. & Chasseaud, L. F. (1969) *Adv. Enzymol. Relat. Areas Mol. Biol.* **32**, 172-219
- Boyland, E. & Williams, K. (1965) *Biochem. J.* **94**, 190-197
- Darby, F. J. & Grundy, R. K. (1972) *Biochem. J.* **128**, 175-177
- Fjellstedt, T. A., Allen, R. H., Duncan, B. K. & Jakoby, W. B. (1973) *J. Biol. Chem.* **248**, 3702-3707
- Fleischner, G., Robbins, J. & Arias, I. M. (1972) *J. Clin. Invest.* **51**, 677-684
- Gelboin, H. V. & Blackburn, N. R. (1964) *Cancer Res.* **24**, 356-360
- Grundin, R., Jakobsson, S. & Cinti, D. L. (1973) *Arch. Biochem. Biophys.* **158**, 544-555
- Habig, W. H., Pabst, M. J., Fleischner, G., Gatmaitan, Z., Arias, I. M. & Jakoby, W. B. (1974a) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3879-3882
- Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974b) *J. Biol. Chem.* **249**, 7130-7139
- Johnson, M. K. (1966) *Biochem. J.* **98**, 44-56
- Kaplowitz, N., Percy-Robb, I. W. & Javitt, N. B. (1973) *J. Exp. Med.* **138**, 483-487
- Kaplowitz, N., Kuhlenskamp, J. & Clifton, G. (1974) *Clin. Res.* **22**, 362A
- Kaplowitz, N., Kuhlenskamp, J. & Clifton, G. (1975) *Biochem. J.* **146**, 351-356
- Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658-666
- Litwack, G., Ketterer, B. & Arias, I. M. (1971) *Nature (London)* **234**, 466-467
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Nebert, D. W., Gielen, J. E. & Goujon, F. M. (1972) *Mol. Pharmacol.* **8**, 651-666
- Ohno, S., Dofuku, R. & Tetterborn, U. (1971) *Clin. Genet.* **2**, 128-140
- Pabst, M. J., Habig, W. H. & Jakoby, W. B. (1973) *Biochem. Biophys. Res. Commun.* **52**, 1123-1128
- Pabst, M. J., Habig, W. H. & Jakoby, M. J. (1974) *J. Biol. Chem.* **249**, 7140-7150