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PULMONARY XENOBIOTIC CONJUGATION IN THE ISOLATED PURFUSED RABBIT LUNG AND *IN VITRO*: EFFECT OF ETHANOL

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ABSTRACT: *Pulmonary conjugation pathways may be important for the metabolism of xenobiotics introduced via airways or systemically. The objective of this study was to determine the pulmonary conjugating capacity in both the isolated perfused rabbit lung (IPRL) and in vitro, and the ability of ethanol to alter the above. The IPRL was capable of conjugating glutathione (GSH) with either 1-chloro-2,4-dinitrobenzene (CDNB) or 1,2-epoxy-(p-nitrophenoxy) propane (ENP). The pulmonary GSH conjugation with ENP was inhibited by cibacron blue, indicating the presence of glutathione-S-transferase (GST) μ and/or π classes, but it was not altered by buthionine sulfoximine, a selective inhibitor of γ-glutamylcysteine synthetase. Neither glucuronide nor sulfate conjugates of 1-naphthol (1-NT) were found in the IPRL. About 35% of the initial 7-hydroxycoumarin (7-HC) was conjugated, the majority being sulfate conjugate (14.4 nmoles/hr) with minor amount (0.12%) of the glucuronide. In vitro, glucuronidation with 1-NT, 7-HC, 4-nitrocatechol and phenolphthalein and sulfation with 1-NT in rabbit lungs were 20 to 40% of those determined in the liver. Acute ethanol treatments decreased the maximal rate of GSH conjugation with CDNB, whereas chronic treatment increased it. None of these ethanol treatments altered GSH conjugation with ENP or pulmonary GSH content. In in vitro studies in rats, acute ethanol administration decreased hepatic GSH content and GST activity in both liver and lung, which were accompained by increased plasma GSH content and GST activity. Chronic ethanol treatment increrased hepatic GSH content. No simple link between alteration in GST activity and lipid peroxidation and/or induction of microsomal enzyme activity was identified. Ethanol did not alter glucuronidation or sulfation in vitro in either liver or lung.*

Key words: *Glutathione conjugation, Glucuronidation, Sulfation, Ethanol, Isolated Perfused Rabbit Lung, in vitro*

INTRODUCTION

The conjugation of xenobiotics, metabolic products of mixed function oxidases and endogenous compounds, is of major importance. For the first two types of reactions conjugation is often responsible for detoxification. Glutathione (GSH) conjugation leading to mercapturic acid formation, glucuronidation and sulfation are the most important pathway in mammalian liver, and a great deal of information has been obtained regarding the distribution and multiplicity of the conjugating enzymes. The conjugating enzymes both glutathione-S-transferase (GST) and UDP-glucuronyltransferase (UDPGT) have been reported to be induced in liver by phenobarbital and 3-methylcholanthrene, which are the classic inducers of cytochrome P450.

Although specific enzyme activities are lower in lung than in liver, conjugations do occur. Since the lungs are situated in a strategic site for exposure to xenobiotics both externally via the inspired air and internally via the circulation, the pulmonary conjugation reactions may have an important function in the detoxification of those xenobiotics.

Ethanol, a widely ingested xenobiotic, alters the activities of various hepatic drug-metabolizing enzymes. The effect of ethanol on hepatic GSH conjugation following either acute or chronic administration have been studied. The results depend on the level and the duration of ethanol consumption. Acute ethanol administration inhibited GSH conjugation in the liver, whereas chronic administration induced it. No studies have been done with regard to the lung. In addition, the inducibility of UDPGT and sulfotransferase (ST) by ethanol has not been fully determined in either liver or lung.

The objective of this investigation was to examine the role of the lung in the conjugation of xenobiotic both quantitatively and qualitatively, using the isolated perfused rabbit lung (IPRL) preparation. The IPRL allows for the evaluation of pulmonary xenobiotic metabolism without interference from extrapulmonary xenobiotic metabolism and also permits better extrapolation to the *in vivo* situation than do studies on subcellular fractions.

MATERIALS AND METHODS

Animals

Male, New Zealand rabbits (Indian Creek, Lafayette, IN) weighing 2-3 kg were housed individually in stainless steel cages. Food (Wayne Rabbit Ration, Wayne Feed, Chicago, IL) and water were allowed *ad libitum*. Male, Sprague-Dawley rats (Harlan Sprangue-Dawley Inc. Indianapolis, IN) weighing 200-300g were used. Rats receiving i.p. treatments were housed in community stainless steel cages (6 to 7 rats per cages). Rats used in the ethanol-drinking studies were housed

individually in stainless steel cages. Food (Purina Lab. Chaw, Ralston Purina Co., St. Louis, MO) and water were allowed *ad libitum*. Light were on a 12 : 12 hr light : dark cycle with the temperature maintained at 21°.

Isolated Perfused Rabbit Lung (IPRL) Preparation

The lungs were surgically isolated and prepared for perfusion in an artificial thorax as previously described (Yang and Carlson, 1990a). Control experiments were conducted to determine the loss of compound to the perfusion apparatus by performing experiments in the usual manner but without the lung. Visual assessment of lung edema, which is readily apparent when it does occur, were made during perfusion for evaluation of intergrity of the pulmonary ultrastructure (Mehendale *et al.*, 1981).

Conjugation Reactions in the IPRL

(1) Glutathione conjugation

The experiment were initiated by addition of either 1,2-epoxy-(*p*-nitrophenoxy) propane (ENP, Sigma, St. Louis, MO) or 1-chloro-2,4-dinitrobenzene(CDNB, Aldrich Chemical Co., Milwaukee, WI) into the perfusion buffer. Aliquots of perfusate were removed at fixed time intervals during the perfusion and were analyzed by HPLC. The concentrations of the substrate and their GSH conjugates were determined by a modification of the method by Sugimoto *et al.* (1985). The perfusate samples were analyzed on a Beckman 332 gradient liquid chromatograph equipped with a Backman 160 absorbance detector set at 340 nm. Aliquots (100 μl) were eluted through a 4.6×4.5 mm Altex Ultrasphere-ODS guard column and 4.6×250 mm Altex Ultrasphere-ODS reverse column. The flow rate was 1.0 m*l*/mm.

For assay of ENP and its metabolites, gradient elution was used. Mobile phases A with 0.1% trifluoroacetic acid (TFA) and B with 50% acetonitrile in programmed from an initial concentration of 60% B to 100% B in 10 min followed by 100% B for 5 min. Then, the system was programmed back to 60% B over 5 min.

A gradient elution system was also used for quantification of CDNB and its GSH conjugate. Mobile phase A was 0.1% TFA, and B was 75% acetonitrile in 0.1% TFA. The gradient elution system was programmed as follows: 1 min isocratic 40% B, followed by 10 min gradient from 40% to 60% B and then a 4 min gradient from 60% to 100% B. The 100% B solvent eluted for an additional 6 min at which point the system was programmed back to 40% B over the span of 4 min.

(2) Glucuronidation and Sulfation

Either 1-[$1-^{14}C$]-naphthol (1-NT, 0.25 or 0.5 mM, 9.4 μCi/mmol, Sigma Chemical Co., St. Louis, MO) or 7-hydroxycoumarin (7-HC, 0.1 mM) was used in the perfusion medium. Aliquots of perfusate were removed at fixed time intervals during the perfusion and were analyzed for quantification of substrates and conjugates by the following methods. The determination of 1-NT and its conjugates in perfusate samples were performed by radioassay by the method of Bock and White (1974). The metabolism of 1-NT in the IPRL was also studied in separate experiments by thin-layer-chromatographic (TLC) separation of 1-NT and metabolites followed by radioassay (Metha *et al.*, 1978). The possible

deconjugation of 1-naphthyl glucuronide (1-NG) and 1-naphthyl sulfate (1-NS) were measured with a modification of Koster and Noordhook (1983). 7-HC conjugation was measured using the procedure of Reinke *et al.* (1986).

Conjugation Reactions in Subcellular Fractions

Subcellular fractions were prepared by homogenizing the liver or lung in 0.1 M Tris buffer containing 1.15% KCl (pH 7.5). The homogenate was centrifuged at 10,000g for 20 min. The supernatant was further centrifuged at 104,000 g for 60 min. The resulting soluble supernatant (cytosolic) fraction was collected, and the microsomal pallet was resuspended in 1 M phosphate buffer (pH 7.25) containing 10 mM EDTA (pH 7.5), 20% glycerin, 0.25 mM phenylmethylsulfonyl fluoride and 0.1 mM dithiothreitol. The preparations were stored at $-70°C$ until assayed.

Glutathione-S-transferase (GST), activities were measured kinetically with 1-chloro-2,4-dinitrobenzene (CDNB), and 3,4-dichloro-1-nitrobenzene (DCNB) according to Habig *et al.*, (1974). Microsomal UDP-glucuronyltransferase (UDPGT) activities were assayed by published methods using the following substrates; 1-NT (Bock and White, 1974), 7-HC(Reinke *et al.*, 1986), 4-nitrocatechol (4-NC, Sweeny and Reinke, 1987) and phenolphthalein (PHPH, Mulder, 1971). Cytosolic sulfotransferase (ST) activity was determined using 1-NT as the substrate.

Other Assays

GSH content was assayed in plasma and tissue using 5,5′-dithiobis-(2-nitrobenzoic acid according to the method of Spiesky *et al.,* (1988). Cytochrome P450 in liver was determined by the method of Omura and Sato (1964). To rule out interference hemoglobin and/or cytochrome oxidase, cytochrome P450 in the lung was determined by the method of Johnnesen and Depierre (1978), which utilizes the difference between oxidized-CO-bound and reduced-CO-bound cytochrome. Hepatic lipid peroxidation was determined as the formation of thiobarbituric acid-reactive products according to the method of Uchiyama and Mihara (1978). Protein concentrations were determined according to the method of Lowry *et al.*, (1951).

Statistical Analysis

Data are expressed as means$\pm$SEM. Statistical comparison of the treatment group with its control was made using Student's *t*-test. When there was more than one treatment group, a one-way ANOVA was used to compare the treatment groups to control, and Duncan's new multiple range finding test (Duncan, 1955) was used to evaluate differences among the group means.

RESULTS

1,2-Epoxy-(*p*-nitrophenoxy)propane is metabolized via GSH conjugation to either 1-(4-nitrophenoxy)-3-(S-glutathionyl)-2-propanol or 3-(4-nitrophenoxy)-2-(S-glutathionyl)-1-propanol in the IPRL. Preliminary studies with an initial concentration of 500 μM ENP indicated that these GSH conjugations were the

only major routes of ENP metabolism in the IPRL, and that ENP metabolism followed linear kinetics at 500 μM ($r = 0.92$). The rate constants for the ENP metabolism of ENP were generated from the slopes of log [ENP] versus time plot. There was no loss of ENP to the perfusion system when perfusate containing ENP was circulated through the tubing and glassware of the IPRL for 60 min in the absence of a lung.

Two experiments were carried out to study the effects of agents which alter GSH synthesis and utilization on GSH conjugation with ENP in the IPRL. The lungs were perfused with buffer containing either a GSH depletor or a GST inhibitor along with the substrate ENP. Buthionine sulfoximine, which selectively inhibits both glutamine synthetase and γ-glutamylcysteine synthetase, produces GSH deficiency without apparent toxicity (Griffith, 1981). The rate of ENP metabolism in the lungs exposed to 0.1 mM buthionine sulfoximine *in vitro* in the IPRL was not significantly different from that of control (Table 1). Various GST isozymes in lung have been characterized in mice (Singh *et al.*, 1987), rats (Robertson *et al.*, 1986) and rabbits (Serabjit-Singh and Bend, 1988). Some similarities in substrate and inhibitor specificity have been proposed as criteria for classification, although substrate/inhibitor specificities overlap to some degree. The specific activities of the purified isozymes towards ENP have been studied (Singh *et al.*, 1987) and GST IV (pI 6.4) and GST VI (pI 4.9) expressed the two highest activities with ENP among all the isozymes of mouse lung. Cibacron blue (1 μM) was chosen as an inhibitor for GST because it inhibits most effectively the near-neutral ($I_{50} = 0.05$ μM) and acidic ($I_{50} = 0.5$ μM) transferase (Tahir *et al.*, 1985). Concurrent administration of cibacron blue resulted in a 66% decrease in GSH conjugation with ENP in the IPRL.

The effects of ethanol on GSH conjugation with ENP were determined. The rate of GSH conjugate formation with ENP in lungs exposed to 0.2% ethanol *in vitro* in the IPRL was not significantly different from that of control. Neither the administration a single dose of ethanol nor the prolonged administration in drinking water altered the rate of GSH conjugation with ENP (Table 2).

The effects of ethanol on GSH conjugation with CDNB, another widely used substrate, were determined. Preliminary studies showed that there were loss of CDNB to the perfusion system and its GSH conjugate, S-(2,4-dinitrophenyl)

Table 1. GST activity with ENP as the substrate in the IPRL

Treatment	(n)	Rate constant (hr^{-1})±SE
Control	5	1.85±0.08
BSO 0.1 mM	2	1.46±0.09
CB 1 μM	2	0.63±0.28*

ENP (500 mM) was perfused through the IPRL for 1 hour. The treated lungs were perfused with buffer containing either 0.1 mM buthionine sulfoximine (BSO) or 1 μM cibacron blue (CB) along with the substrate ENP. Rate constant were generated from log [ENP] vs. time plots. Values represent means±SE.

*Significantly different from control ($P<0.01$).

Table 2. Ethanol effects on GST activity with ENP as the substrate in the IPRL

Treatment	Rate constant (hr^{-1}±SE)
control	1.36±0.05
0.2% Ethanol	1.57±0.13
Acute	1.20±0.07
Chronic	1.21±0.17

Control lungs were perfused with Kreb-Henseleit buffer. The rabbits in the 0.2% ethanol received water but the lungs were perfused with buffer containing 0.2% ethanol. Rabbits in acute group received a single dose of ethanol (4 g/kg by gastric intubation) 4 hour before the IPRL preparation, and those in the chronic group received 10% (w/v) ethanol in drinking water for 3 weeks. No significant differences in the rate of GSH conjugation were found among the groups (P>0.05).

glutathione (DNPG) to tissue (Yang and Carlson, 1990b). The effects of ethanol on GST activity with CDNB, therefore, were evaluated by determining the initial rate of DNPG formation as a function of initial CDNB concentration tested (100-1000 μM). This procedure provides considerable simplification in the case of reversible reactions or in cases where side reactions occur as the reaction proceeds (Moore and Pearson, 1981). The effects of ethanol *in vitro* and following a single dose *in vivo* on GSH conjugation with CDNB were studies. When the initial rates of DNPG formation were plotted as a function of the intial CDNB concentration used, they appeared to depend on the initial CDNB concentration at low concentrations (100-400 μM) (Fig. 1A). The production of DNPG plateaued at high initial CDNB concentrations (500-1000 μM). A single dose of ethanol *in vivo* (4 g/kg body weight by gstric intubation) or *in vitro* administration (0.2% ethanol in the perfusion buffer) had no effect on the rate of GSH conjugation with CDNB at low concentrations, but caused decreases in the maximal rate of GSH conjugation compared to control in the IPRL. The inducibility of GSH conjugation with CDNB in the lung was studies in rabbits given 10% (w/v) ethanol in drinking water for 3 weeks which gave rise to an average blood alcohol content of 0.012%. In contrast to the results obtained following acute ethanol administration, the rate of DNPG formation was increased in the lungs from ethanol-treated rabbits at all concentrations of CDNB tested (Fig. 1B).

Upon termination of perfusion, the GSH levels in lungs from controls and rabbits treated with ethanol *in vitro*, acutely and chronically were determined to evaluate changes in this co-substate, if any. There were no significant differences among the groups.

The effect of ethanol on GSH conjugation was further studied in the subcellular fraction with respect to the dose and duration of ethanol administration. A single dose of ethanol (3 g/kg) caused a significant (P<0.05) decrease in hepatic GSH from 7.00±0.24 μmol/g tissue to 4.17±0.34 μmol/g tissue, whereas no changes were observed in the lung (control: 1.56±0.24 μmol/g tissue and ethanol treated 1.60±0.24 μmol/g tissue) after 4 hr. Hepatic GSH has an especially important relationship with lipid peroxiation because of its albility to bind with free radicals

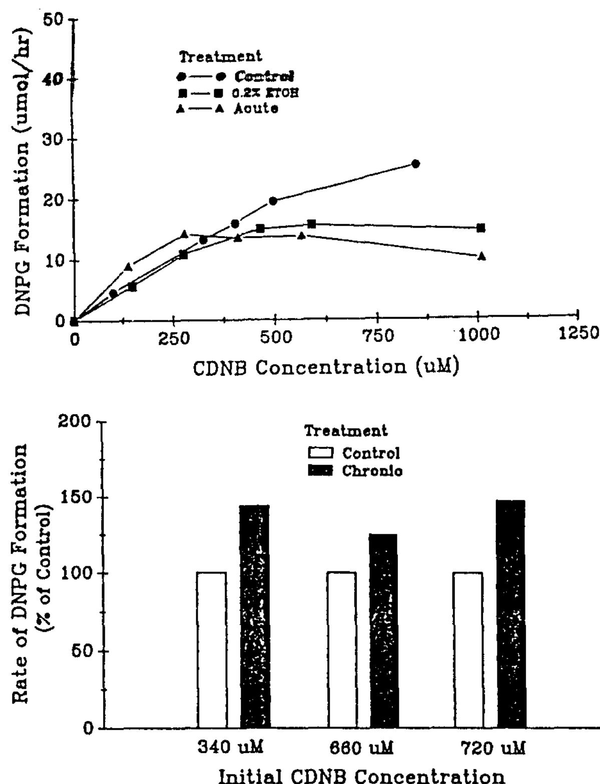

Fig. 1. Effects of ethanol on GST activity in the IPRL. Lungs were perfused with varying initial concentrations of CDNB for 45 min. The ordinate shows DNPG formation (μmol/h) and the abscissa gives the initial CDNB concentrations in the recirculating perfusate. (A) Control received water and the acutely dosed rabbits received a single dose of ethanol (4g/kg by gastric intubation) 4 hr before the IPRL preparation. The rabbits in the 0.2% ETOH group received water, but their lungs were perfused with buffer containing 0.2% ethanol. Each point represents a value obtained from a single experiment. (B) Control rabbits received water and the chronic group received 10% (w/v) ethanol in drinking water for 3 weeks. Each bar represents a value obtained from a single experiment.

that may initiate peroxidation (Farooqui and Ahmed, 1984; Kawase *et al.*, 1989). Hepatic lipid peroxidation in ethanol treated rats showed a 44% increase in absorbance (535-600 nm) from 0.09 to 0.14, although the differences was not statistically significant because of the large variation among animals. The activity of GST with CDNB as the substrate was decreased in liver and lung from acutely reated rats (Fig. 2), but enzyme activity with DCNB was not altered. Hepatocyte GSH easily passes into the plasma following injury (Botti *et al.*, 1982), and plasma GSH is increased by acute administration in rats (Fernandez *et al.*, 1983). Therefore, plasma GST activities as well as GSH concentrations were measured to evaluated translocation processes from liver to plasma. The acute treatment with ethanol resulted in a 34% increase in plasma GSH from 4.05±0.73 nmol/m*l*

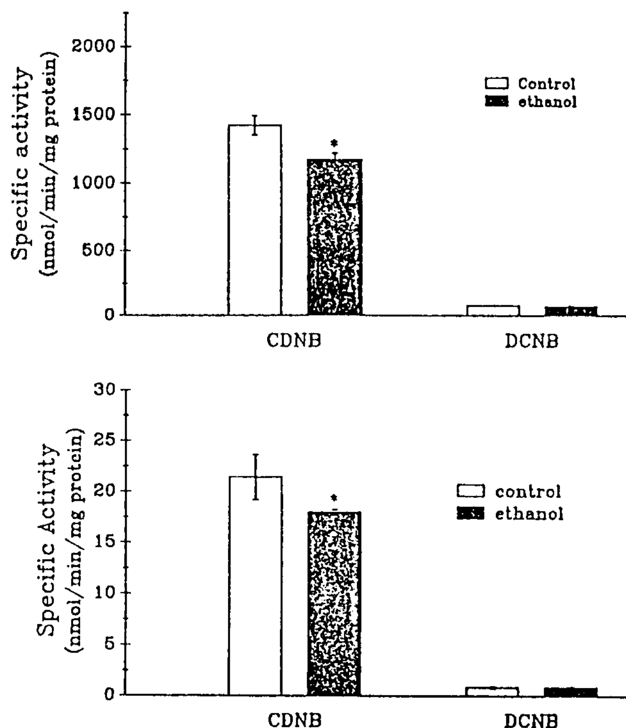

Fig. 2. Effect of acute ethanol administration on GST activity. In the ethanol treatment group (n=6), the rats received 4g/kg body weight, i.p., 6 hr before decapitation. Controls (n=6) received saline. The GST activities are (A) Liver, (B) Lung. The vertical lines indicate the SEM. *Significantly different from control ($P<0.05$).

to 5.44±1.15 nmol/m*l* and a 39% increase in CDNB-conjugating activity from 58.3±3.22 nmol/m*l* to 80.8±6.87 nmol/m*l*.

The effects of chronic ethanol were studied in rats received ethanol in drinking water at concentrations of 5 and 10% for 3 weeks. Hepatic GSH in rats drinking ethanol increased in a dose-dependent manner (Fig. 3). PB and βNF also increased hepatic GSH, but the induction was less than in rats treated with 10% ethanol. Prolonged ethanol administration caused no significant increase in lipid peroxidation. Only with βNF caused hepatic lipid peroxidation (data not shown). Chronic ethanol administration caused no significant induction in GST activity in either organ (Table 3). In liver, PB induced GST activities with both substrates. In contrast to the effects of acute ethanol adminitration, chronic ethanol administration (10%) diminished plasma GST activity (Fig. 4). Neither PB nor βNF affected plasma GST activity. There was no increase in cytochrome P450 in either organ following chronic ethanol administration (Table 3). PB and βNF caused 178 and 68% increases.

The conjuagtion of 1-NT was studies in the IPRL in perfusion lasting 90 min. With 0.25 and 0.5 mM 1-NT, virtually no glucuronidation or sulfation occurred (Fig. 5A). An initial rapid decrease of 1-NT in the perfusate (about 20% of the

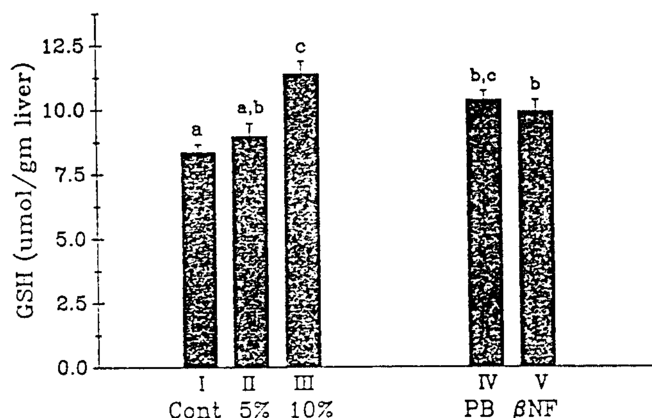

Fig. 3. Effect of chronic ethanol administration on hepatic GSH concentration. Controls (group I, n=6) drank tap water, Group II (n=6) and group III rats (n=6) received 5 and 10% ethanol (w/v) in drinking water for 3 weeks, respectively. Group IV rats (n=4) were injected i.p. with PB (80 mg/kg body weight) and group V rats wer injected i.p. with βNF (40 mg/kg body weight) daily for 3 days, Six samples were pooled. Data are means±SEM of two or three pools. [a-c]Values within the same column with different superscripts are significantly different ($P<0.05$).

Table 3. Effect of ethanol, phenobarbital (PB), and β-naphthoflavone (βNF) on cytochrome P450 and cytosolic and microsomal GST activities in rat

Group	Cytochrome P450 (pmon P450/mg protein)	Glutathione-S-transferase (nmole/min/mg protein)	
		CDNB	DCNB
(A) Liver			
I. Control	683± 88^{a}	1375±66^{a}	65±2^{a,b}
II. Ethanol (5%)	897±107^{a,b}	1382±94^{a}	65±3^{a,b}
III. Ethanol (10%)	800± 54^{a,b}	1581±90^{a}	71±4^{b}
IV. PB	1904±320^{c}	1907±78^{b}	89±4^{c}
V. βNF	1105± 77^{b}	1630±63^{a}	59±5^{a}
(B) Lung			
I. Control	41±4^{a}	32±1.3^{a}	1.2±0.1^{a}
II. Ethanol (5%)	47±2^{a}	34±1.5^{a}	1.1±0.1^{a}
III. Ethanol (10%)	46±4^{a}	35±1.2^{a}	1.3±0.2^{a}
IV. PB	48±10^{a}	30±1.4^{a}	1.1±0.1^{a}
V. βNF	82± 9^{b}	33±4.6^{a}	1.1±0.1^{a}

Controls (group I, n=6) drank tap water. Group II (n=6) and group III rats (n=6) received 5 and 10% ethanol (w/v) in drinking water for 3 weeks, respectivley. Group IV rats (n=4) were injected i.p. with PB (80 mg/kg body weight) and group V rats were injected i.p. with βNF (40 mg/kg body weight) daily for 3 days, Six samples were pooled. Data are means±SEM of two or three pools. [a-c]Values within the same column with differnt superscripts are significantly different ($P<0.05$).

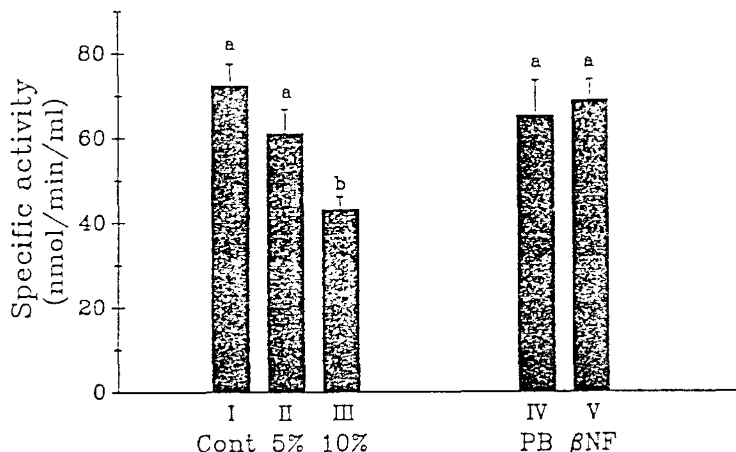

Fig. 4. Effect of chronic ethanol administration on plasma GST activity. Controls (group I, n=6) drank tap water. Group II (n=6) and group III rats (n=6) received 5 and 10% ethanol (w/v) in drinking water for 3 weeks, respectively. Group IV rats (n=4) were injected i.p. with PB (80 mg/kg body weight) and group V rats were injected i.p. with ωNF (40 mg/kg body weight) daily for 3 days, Six samples were pooled. Data are means±SEM of two or three pools. [a-c]Values within the same column with different superscripts are significantly different (P<0.05).

initial concentration) was detected within 5 min. This was not due to the loss of 1-NT to the lung perfusion system, since it was not observed in the absence of lung. To examine the possibility that other metabolites of 1-NT were being produced in the lung, the perfusate was analyzed using TLC to quantitatively measure the metabolites present in the perfusate. The chromatographic analysis indicated that neither other metabolites nor glucuronide and/or sulfate were formed in the IPRL (Fig. 5B). Failure to detect glucuronidation and sulfation by the lung could conceivably be due to high level of pulmonary β-glucuronidase and sulfatase. The de-conjugations of 1-NG and 1-NS in the IPRL were determined by measuring the amout 1-NT liberated during 90 min or perfusion with these conjugates. No detectable levels of 1-NT were linerated from either conjugate (data not shown).

Glucuronidation and sulfation as well as effect of ethanol in the subcellular fractions were determined (Table 4). Pulmonary microsomes from rabbits exhibited significant UDPGT activity. This activity was approximately 20-50% of that in the liver. UDPG activity with 1-NT was measured by the naive (nonactivated) microsomes in order to reveal any possible latency of the UDPGT activity. UDPGT activity in naive microsomes was 67% of that in Brij 58-activated microsomes in both lung and liver. In contrast to reports of increased UDPGT activity following ethanol administration (Hutabarat and Yost, 1989, Hutabarat *et al.*, 1989), no significant induction by ethanol was found. The activity of hepatic and pulmonary ST was determined (Table 4). In aggreement with others (Reinke *et al.*, 1986), ethanol had no influence on ST activity in the liver. It also did not affect ST activity

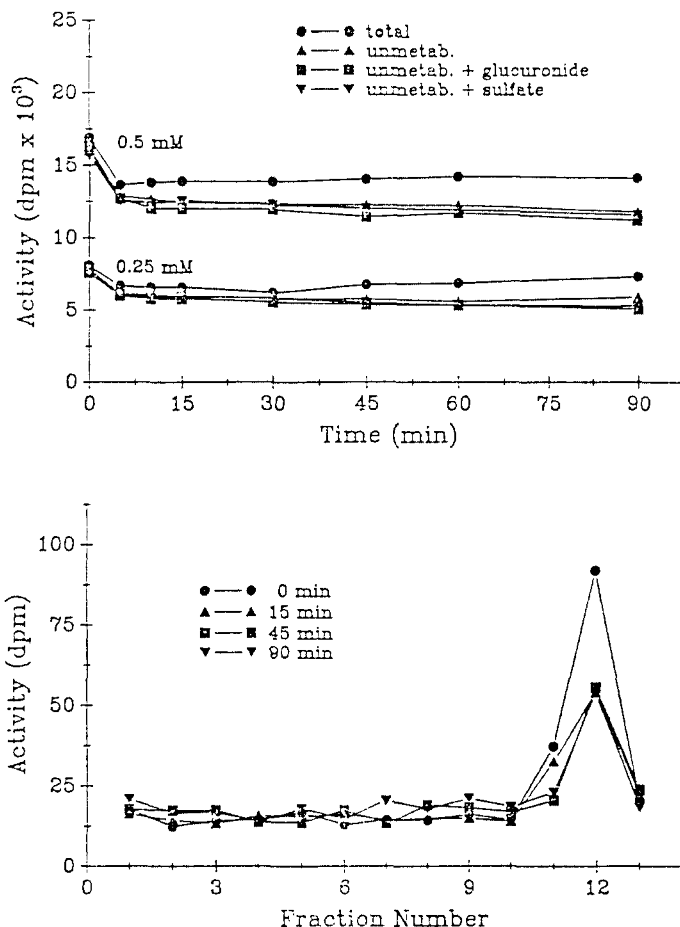

Fig. 5. Metabolism of 1-NT in the IPRL. (A) Rabbit lungs were perfused with two concentrations of 1-NT. Samples of perfusate were obtained at various times, and the radioactivities determined for total, unmetabolized 1-NT plus 1-NG and unmetabolized 1-NT plus 1-NS. Data represent individual experiments. (B) 0.5 mM 1-NT was circulated through the IPRL. Samples of perfusate were obtained at various times followed by TLC separation and scintillation counting of fractions scraped from the plates. The R_f values were 0.13 (fraction 2), 0.5 (fraction 7) and 0.8 (fraction 11) for 1-NG, 1-NG and free 1-NT respectively.

in the lung. The conjugation of 7-HC, another commonly used substrate, with glucuronide and sulfate was studied in the IPRL. The concentration-time profile for the IPRL with 0.1 mM 7-HC is shown in Fig. 6. Only 70% of the initial concentration of 7-HC was detected as either 7-HC or its conjugates in the perfusate after 150 min. The appearance of metabolites was approximately linear over 150 min (r=0.97). About 35% of inital 7-HC was conjugated, the majority being sulfate conjugate (14.4 nmol/h) with only minor amounts (0.12%) of the glucuronide conjugate.

Table 4. Hepatic and pulmonary UDPGT and ST activities in rabbits

Substrate	Liver		Lung	
	Control	Ethanol	Control	Ethanol
UDPGT activity				
Activated microsomes[1]				
1-NT	33.2±0.7	38.0±9.5	9.2±1.3	7.8±1.3
7-HC	5.2±0.4	6.2±0.5	1.2±0.2	1.5±0.1
4-NC	0.8±0.3	1.4±0.9	0.5±0.1	0.5±0.1
PHPH	2.1±0.4	3.1±0.5	1.1±0.5	1.0±0.3
Nonactivated microsomes[2]				
1-NT	22.1±0.4	25.6±9.0	6.2±0.9	6.2±0.6
ST activity				
1-NT	7.0±0.8	7.0±0.8	2.8±0.2	3.3±0.8

Values are mean±S.E. No significant differences from control were observed (P>0.05). Ethanol treated rabbit (n=4) drank 10% ethanol in drinking water for 2 weeks. Control received water (n=4).

[1] Microsomes were activated with 0.25% Triton X-100, except 1-NT where Brij 58 was used. [2] UDPGT activity was measured in nonactivated microsomes. No significant difference was found between treated and control (P>0.05).

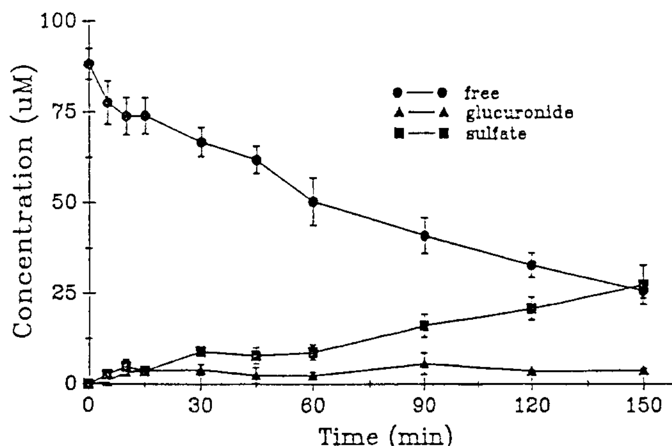

Fig. 6. Glucuronidation and sulfation of 7-HC. 0.1 mM 7-HC was perfused in rabbit lungs. Samples of perfusate were obtained at various times, and the concentrations of unmetabolized 7-HC and glucuronide and sulfate conjugates were determined. The values represent the means±SE of 4 experiments

DISCUSSION

The ability of the lung to conjugate xenobiotics was investigated in the IPRL and *in vitro*. The rabbit lung as assessed in the IPRL preparation was capable of conjugating GSH with either CDNB or ENP. The IPRL was also capable of

detecting change in GSH conjugation. Cibacron blue, which effectively inhibits the near-neutral and acidic forms of the transferases, extensively diminished GST activity in the IPRL. The extensive inhibition by cibacron blue indicates the presence and importance of GST μ and/or GST π classes in the rabbit lungs. On the other hand, lungs perfused with the γ-glutamylcysteine synthetase inhibitor buthionine sulfoximine did not show an alteration in the rate of GSH conjugation with ENP. Griffith and Miester (1979) demonstrated a decrease in the GSH levels in the liver soon after subcutaneous administration of buthionine sulfoxime but not in the lung. Lung has a lower turnover rates of GSH when compared to liver (Griffith and Miester, 1979). Cells that have low rates of turnover might be depleted of GSH first with a substrate that reacts with GSH to form a conjugate and then with an inhibitor of GSH synthesis. This may make GSH levels in the lung less susceptable to alteration by buthionine sulfoximine.

Glucuronidation and sulfation with 1-NT and 7-HC were also studied in the IPRL. Surprisingly neither glucuronide nor sulfate conjugates on 1-NT were found. The absence of conjugate formation was not due to the presence of β-glucuronidase and/or sulfatase or due to alternative biotransformation pathways in the lung. When 7-HC was studies, about 35% of the initial compound was conjugated, the majority being sulfate with only minor amounts of the glucuronide. However, when glucuronidation and sulfation were studied in subcellular fractions *in vitro,* the presence of UDPGT activity with 1-NT, 7-HC, 4-NC and PHPH and ST with 1-NT in rabbit lungs were substantiated. The activities were approximately 20% to 40% of those determined in the liver. Reinke and associates (1986) demonstrated that the UDP-glucuronic acid from carbohydrate reserves was the rate-limiting factor for glucuronidation in the isolated perfused rat liver. The lack of glucuronidation in the IPRL despite its presence *in vitro*, may be due to the availability of UDP-glucuronic acid in the lung. These results inducate the importance of studying both whole organ and *in vitro* metabolism.

Studies on the inducibility of UDPGT by ethanol have shown conflicting results. Ethanol (10% in drinking water for 2 weeks) administration resulted in a significant increase in the rabbit liver microsomal UDPGT activities with 1-NT, morphine and *p*-nitrophenol (Hutabarat and Yost, 1989). Yost and associate (1989) reported that in the rabbit liver, ethanol (10% in drinking water for 2 weeks) was a better inducer of *p*-nitrophenol UDPGT activity than was PB or 3-methylcholanthrene. In contrast with those reporting inducibility by ethanol, our results showed that ethanol did not induce DUPGT to any great extent. However, they are consistent with previous work in this laboratory (Trela and Carlson, 1985; Yang and Carlson, 1991a) and in aggreement with those of others (Dutton, 1980; Hiethanan *et al.*, 1980). Dutton (1980) suggested that ethanol does not induce UDPGT because of its rapid metabolism and clearance.

The inducibility of GSH conjugation by ethanol was studied in IPRL system with particular attention paid to the duration and route of ethanol administration. As expected from the results obtained with buthionine sulfoximine treatment, GSH levels in the lung were not altered by either acute or chronic ethanol administration. The maximum rate of the GSH conjugation with CDNB decreased in rabbits treated acutely with ethanol or when ethanol was added *in vitro*

indicating that ethanol inhibits the GSH-conjugating system in the IPRL when present at high concentrations. On the other hand, the GSH-conjugating system in the lung induced in rabbits treated for 3 weeks with ethanol in the drinking water. These findings are in agreement with previous reports on the liver (Kocak-Tokey *et al.*, 1985; Aykac *et al.*, 1985). In contrast to the results with CDNB, none of the ethanol treatments altered GSH conjugation with ENP. Differential effects of ethanol on GST activities depending upon the substrate have been demonstrated in rats (Hetu *et al.*, 1982) and mice (David and Nerland, 1983). Since there were no differences in pulmonary GSH concentrations between control and ethanol-treated groups, the possibility of an altered GSH level affecting the rate of GSH conjugation might be excluded as an explanation for the effects of ethanol on the rate GSH conjugation.

In attempt to understand the possible mechanism responsible for the effect of ethanol on GSH conjugation, the effect of ethanol on the GSH conjugation system and its correlation with lipid peroxidation and/or induction of microsomal enzymes was investigated *in vitro* in both liver and lung in rat with respect to the dose and duration of ethanol administration. Significant decreases in hepatic GSH content and GST activity in both liver and lung were observed in rats treated acutely with ethanol. This is in aggrement with what has been reported previously (Aykac *et al.*, 1985). The decreases in hepatic GSH content and GST activity upon acute ethanol administration could be due to an increase in the loss of both GSH and GST from liver to plasma. Acute ethanol administration has been reported to cause the release of corticosteroids, glucagon and epinephrine (Speisky *et al.*, 1988; Sies and Graf, 1985), which increase the sinusoidal efflux of GSH from the perfused liver (Spiesky *et al.*, 1988). Since GSH provides several lines of defence against peroxidative damage in connection with GSH-peroxidases, increased lipid peroxidation after acute ethanol administration may deplete hepatic GSH by increasing its utilization.

Chronic administration of ethanol caused dose-dependent increases in hepatic GSH content. GSH synthesis is thought to be regulated by end-product feedback inhibition (Hayes and Wolf, 1989). Although chronic ethanol treatment showed variable results with GST in the present study, it does not rule out the possibility of induction of hepatic GST by chronic ethanol administration under other condition. Hetu *et al.* (1982) deminstrated induction of hepatic GST activities with CDNB and DCNB when ethanol was adminstered as 36% of the total calories in a liquid diet for 3 weeks. Younes *et al.* (1980) reported that rats which received 15% ethanol (v/v) in drinking water for 2 weeks showed increased hepatic GST activity with ENP but not with DCNB. When animals were given 15% ethanol in drinking water, the amount ethanol they consumed amounted to 18% of their total caloric intake, and there was no evidence of a specific toxic effect of ethanol upon liver cell (Lieber *et al.*, 1989). Therefore, 10% ethanol in drinking water might not be sufficient to sustain levels of ethanol in the blood enough to cause significant induction of GST activity.

The inducibility of GST by chronic ethanol treatment has been explained by either an adaptational change in response to ethanol-induced lipid peroxidation

(Aykac *et al.*, 1985; Hetu *et al.*, 1982) or an alteration related to factors which influence microsomal enzyme activity (Younes *et al.*, 1980). However, βNF, which caused a 1.3 fold increase in hepatic lipid peroxidation, did not induce GST activity to any great extent than did ethanol. In addition, the response of GST differs from that of cytochrome P450 (Yang and Carlson, 1991b). In disagreement with Younes *et al.* (1980) who suggested that cytosolic GST activity is subject to alterations due to the action of influence the microsomal enzyme activities, GST activity remained unchanged in both cases. No simple link between lipid peroxidation and/or induction of microsomal enzyme activity and alterations in GST activity was identified.

In conclusion, this investigation has demonstrated that rabbit lungs assessed in both the IPRL and *in vitro* were capable of GSH conjugation. Glucuronidation and sulfation in intact lung cell (IPRL) showed a different profile from that observed in the pulmonary subcelluar fraction preparations. Neither glucuronide nor sulfate conjugates of 1-NT were observed in the IPRL despite its presence *in vitro*, whereas sulfation with very low glucuronidation of 7-HC was detected. These results indicates the importance of studying metabolism in both whole organ and *in vitro* preparation. This study indicates that GSTs, among other conjugating enzymes, might play a major detoxifying role in the lung *in vivo*. Since GST activity was inhibitable in the lung, the effects of chemicals on pulmonary GSH conjugation may be viewed as alterations of the metabolic disposition and toxicity of certain xenobiotics in the lung.

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