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EFFECTS OF 1,2-DIBROMOETHANE ON GLUTATHIONE METABOLISM IN RAT LIVER AND KIDNEY

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Abstract—There are few reports of the effects of glutathione-depleting agents administered for periods longer than 24 hr on the turnover of glutathione (GSH) in mammalian tissues. Studies of such effects are important in relation to the protection of tissues from damage from, for example, reactive metabolites derived from xenobiotics. In the investigation described here, 1,2-dibromoethane (DBE) (ethylene dibromide)—a widely used insecticide, nematocide, fungicide and petrol additive, which is hepato- and nephrotoxic—was administered to rats and the effects on non-protein thiol contents and GSH-related enzyme activities were determined in liver and kidney. The classical GSH-depleter diethylmaleate was used in parallel studies for comparative purposes.

MATERIALS AND METHODS

1,2-Dibromoethane (DBE) was purchased from British Drug Houses (Dagenham, Essex, U.K.) and diethylmaleate (DEM) from Sigma (Poole, Dorset, U.K.). (U-^{14}C) labelled glutamic acid (50 μCi/mmole) and (U-^{14}C) labelled glycine (50 μmCi/mmole) were obtained from Radiochemical Centre (Amersham, U.K.). The γ-μglutamyl transpeptidase kit (no. 416-B) was purchased from Sigma. All other reagents were of commercial AR quality.

Animals. Male and female Wistar rats were purchased from Bantin and Kingman (Aldbrough, Hull, U.K.), and housed in a temperature-, light-cycle (6.00 a.m.–6.00 p.m.) and humidity controlled room and allowed at least 5 days to become acclimatized before use. The animals weighed 200–250 g when used experimentally. DBE in olive oil (10% w/v) was administered i.p. at a dose of 80 mg/kg body wt. DEM was administered similarly at a dose of 400 mg/kg body wt. Control rats received appropriate volumes of olive oil and were maintained concurrently.

NPT determination. The animals were stunned and sacrificed by cervical dislocation at 0, 2, 4, 6, 8, 24 and 96 hr after a single injection of DBE or olive oil and at 24 hr after two, four, six and eight daily injections. The liver was rapidly excised from each animal, weighed, and 1-g portions used for determination of NPT concentrations or water content (determined as weight loss on drying portions of tissue from liver and kidney to a constant weight at 105° [1]).

The wet wt of the kidneys was determined after removal of the capsule and extraneous fat. A portion of left kidney was used for water content determination. The right kidney was used for NPT determination by Ellman's method as modified by Boyland and Chasseaud [2]. Bis-(3-carboxy-4-nitrophenyl) disulphide [3] reacts with non-protein thiols (NPT) in general, however, it is commonly accepted that the main reacting NPT in the tissues is GSH [4]. GSH determined by Ellman's reagent has been found to give results comparable with those of the more specific enzymatic assay of Bernt and Bergmeyer for actual GSH [5].

GSH-related enzymes

Experimental and control male rats were sacrificed 24 hr after four consecutive daily injections of DBE. This time period showed the greatest overshoot in NPT levels in kidney in male rats.

Glutathione (GSSG) reductase; glutathione (GSH) peroxidase. One-gram portions of liver or kidney were homogenised in 9 vol. (w/v) of ice-cold potassium phosphate buffer 5 mmoles/l pH 7.6 containing 1.15% (w/v) potassium chloride, and then centrifuged at 10,000 *g* for 15 min (4°). The resulting supernatant was spun for a further 60 min at 105,000 *g* (4°). This supernatant was assayed for both enzymes. The GSSG reductase assay followed [6] and the GSH peroxidase assay followed Paglia and Valentine [7] as described by Rister *et al.* [8]. In both assays, NADPH disappearance at 340 nm was directly indicative of enzyme activity.

γ-Glutamylcysteine (γ-GC) synthetase; GSH synthetase assays. 1:5 (w/v) homogenates were prepared from liver and kidney using 0.01 moles/l potassium phosphate, pH 7.4, containing 1.15% (w/v) potassium chloride. 105,000 *g* supernatants were prepared as before for the GSSG reductase assay. Assays of both enzymes were measured by following the incorporation of ^{14}C-labelled glutamic acid and ^{14}C-labelled glycine into γ-glutamyl cysteine and GSH respectively [9, 10]. The substrate, γ-glutamyl cysteine, for the GSH synthetase assay was prepared by the method of Strumeyer and Bloch [11].

γ-Glutamyl transpeptidase (γGT). Ten per cent (w/v) liver and kidney homogenates were prepared in 0.05 moles/l potassium phosphate pH 7.4, containing 0.25 M sucrose [12]. γGT activity was determined using Sigma reagent kit no. 416-B, a quantitative kinetic procedure based on the optimised method of Szasz [13]. The 10% homogenates were

adjusted with phosphate buffer, pH 7.4, to give a change in absorbance of about 0.02/min and 0.2/min for liver and kidney extracts respectively.

Protein determination. All protein determinations were carried out using the biuret method, with bovine serum albumin as standard.

RESULTS AND DISCUSSION

Figure 1 confirmed earlier observations by Nachtomi *et al.* [14] that a single dose of DBE depletes NPT from rat liver. The greater depletion in hepatic NPT (Fig. 1) in female animals may result from DBE removing the same molar amount of GSH in males and females; females have a lower normal hepatic GSH content and so exhibit greater percentage depletion. Rapid recovery to control levels was found within 6 hr, and in females, this recovery proceeded to overshoot normal levels in liver significantly at 24 hr. In the same rats, kidney NPT levels were not depleted by DBE at this dose when compared with control animals (Fig. 1). This difference in liver and kidney response to the single injection of DBE has been observed with other halogenated aliphatic hydrocarbons: for example—dibromochloropropane [15] and hexachlorobutadiene [1]. Dose–response investigations, however, have shown that higher doses (above 100 mg/kg i.p.) of DBE could significantly deplete kidney NPT [4]. Presumably, liver would be able to deal with most of the smaller doses, since it is the major site of detoxication, without the kidney being affected. Renal NPT levels in females were significantly higher than controls at 8 and 24 hr post-treatment and in males, at 24 hr, although the dose used caused no depletion of NPT. This phenomenon was also observed 16 hr after a single injection of dibromochloropropane [15] and after hexachlorobutadiene [1].

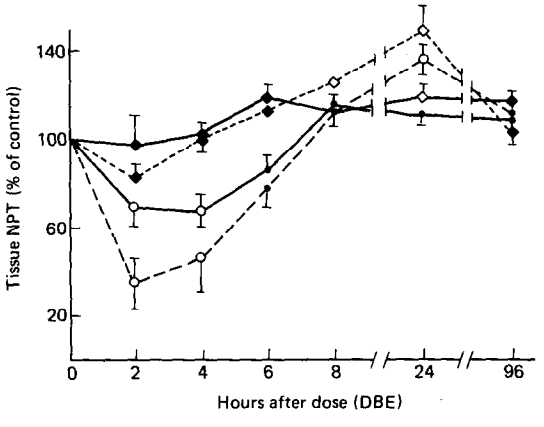

Fig. 1. NPT (GSH) depletion in male (——) and female (– – –) rat liver (●) and kidney (◆) following a single dose of DBE (80 mg/kg i.p.). Each point represents a mean ± S.E.M. of at least four DBE-treated animals as a percentage of NPT (GSH) concentrations found in vehicle-treated animal ($N \geq 4$) at each respective time period. The mean ± S.E.M. of NPT concentration of all control animals (0–96 hr inclusive) was: 6.6 ± 0.3 (males) and 5.9 ± 0.3 (females) μmoles/g wet liver and 2.6 ± 0.1 (males) and 3.7 ± 0.1 (females) μmoles/g wet kidney. Open symbols indicate a significant difference from controls using the Mann–Whitney *U*-test: ($P < 0.05$).

The enhanced NPT levels in both liver and kidney seen after repeated daily injections of DBE (Figs 2a,b) have also been noted in rat liver after chronic exposure to vinyl chloride [16]. DEM also depletes liver NPT within 1 hr of single administration [15]. Rats treated with four consecutive daily injections of DEM, as with DBE, also exhibited transitory peaks in overshoot NPT values, but to a lesser extent. Possibly, these increased NPT levels may be a series of depletions caused by DBE and DEM in this case, followed by rapid resynthesis to above normal tissue concentrations.

Why single or chronic administrations of xenobiotics often results in these overshoot NPT values needs to be clarified. It is unknown whether these enhanced NPT consist of GSH or other non-protein thiols. However, it may be the temporary lowering during chemical conjugation of the GSH feedback inhibition of γ-glutamyl cysteine synthetase [17] which initiates increased GSH synthesis. Non-chemical GSH depletion in rat liver following maintenance on low-protein diets also results in overshoot GSH levels after protein has been restored [18]. This further suggests that reduction of the GSH content of liver will result in an increased rate of resynthesis.

The enzyme studies reported here support the view that there could be increased GSH synthesis in liver arising at least in part from an increase in γ-glutamylcysteine activity (GSH synthetase activity remained normal) in response to four consecutive daily DBE injections (Table 1). Treatment with DEM for 4 days was also associated with enhanced (40%) γ-glutamylcysteine synthetase activity. In the animals treated with DEM, but not DBE, hepatic GSH peroxidase activity was reduced compared with controls (64.0 ± 4.2 U/100 mg protein down to 47.9 ± 3.5; $P < 0.05$) but this would not be thought to be significant in relation to NPT content elevation here. Accompaniment of high GSSG reductase activities by increased NPT content concentrations in rat liver has been observed after chronic administration of vinyl chloride [16] and often increases in GSSG reductase activity have been paralleled by increases in GSH *S*-transferases' activity [16, 19]. It is doubtful whether the increased GSSG reductase activity would have contributed significantly to the high NPT content found in this study as normally GSSG concentrations are low (about 5% of GSH values; [20]). However, increase in activity suggests that this enzyme is compensating for some imbalance in the (GSSG)/(GSH) ratio. Multiple administrations of DBE produced statistically significant increases in the liver:body wt and kidney:body wt ratios of male and female animals (liver, females, 35% increase relative to controls after 4 days of treatment; liver, males, 20% increase: kidney, females, 25% increase; kidney, males 35% increase). DEM was associated solely with an increase in the kidney:body wt ratio of 25% after 4–6 days of treatment. Nachtomi and Farber [21] found an increased liver:body wt ratio 18 hr after a single oral DBE dose (100 mg/kg). (This response varied with dose.) Investigations with hexachlorobutadiene [1] associated such raised tissue:body wt ratios with water retention. The present study, however, revealed no change in the water content (%g H_2O/g wet wt) of

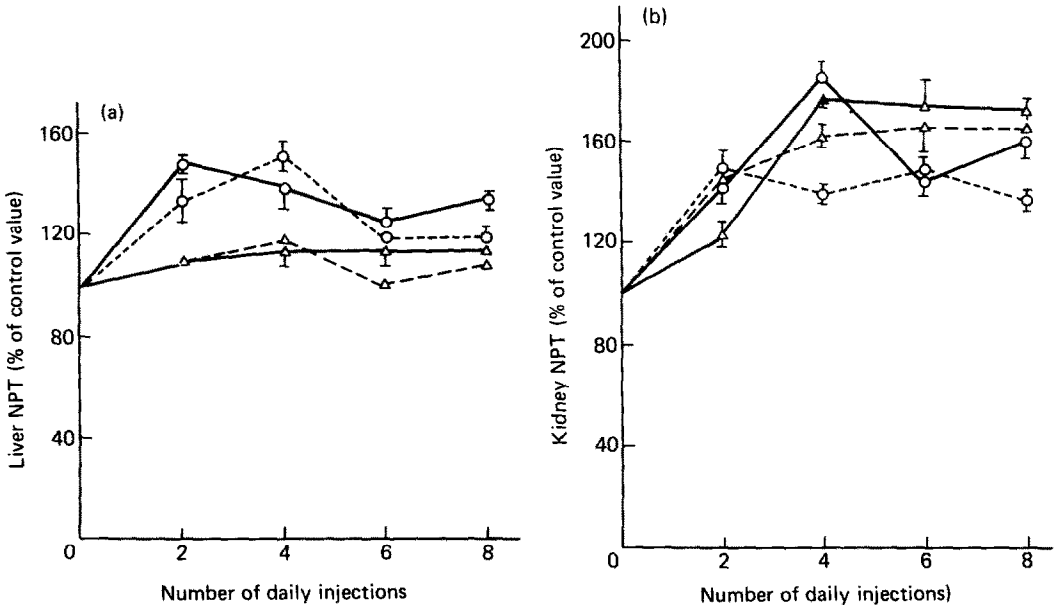

Fig. 2. NPT (GSH) levels in rat liver (graph a) and kidney (graph b) following multiple doses of DBE (○) 80 mg/kg i.p. and DEM (▲) 400 mg/kg i.p. NPT (GSH) values were plotted as a percentage of the control values obtained for rats sacrificed simultaneously at each time period ($N \geq 4$). NPT is expressed as μmoles/g wet tissue in male (——) and female (– – –) animals. The mean ± S.E.M. of NPT concentrations of all control animals (0–8 daily injections inclusive) from DBE and DEM experiments (separate controls) were:- 7.4 ± 0.1 (male) and 6.2 ± 0.5 (female) μmoles/g wet liver and 3.0 ± 0.1 (male) and 3.9 ± 0.2 (female) μmoles/g wet kidney. Open symbols indicate a significant difference from controls using the Mann–Whitney *U*-test ($P < 0.05$).

either organ of DBE or DEM-treated rats (male or female). Increased *de novo* synthesis of general cytosolic proteins was not observed; it is not known if cellular organelles were increased in size.

This report has shown that repeated daily injections of DBE and DEM increase NPT content of both liver and kidney in male and female rats. Studies on the activities of GSH-related enzymes suggest that there may be increased GSH synthesis in liver of both DBE and DEM-treated rats with additional decreased GSH utilization (GSH peroxidase) in DEM-treated animals only, associated with *de novo* synthesis of γ-glutamylcysteine synthetase.

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Table 1. Effect of DBE administration *in vivo* in rat liver and kidney enzyme activities. Groups of at least six male Wistar rats (200–250 g) were treated daily with DBE (80 mg/kg i.p.) or olive oil for 4 successive days and killed 24 hr after last injection. Data are presented as mean ± S.E.M. (standard error of mean)

Organ	Treatment	GSSG reductase (U/100 mg protein)[a]	GSH peroxidase (U/100 mg protein)[a]	γ-GC synthetase (nmoles ^{14}C-gly/ mg protein)[b]	GSH synthetase (nmoles ^{14}C-gly/ mg protein)[b]	γ-glutamyl transpeptidase (U/g protein)[c]
Liver	DBE	*5.2 ± 0.3	72.8 ± 5.7	*12.1 ± 0.9	21.8 ± 1.9	0.74 ± 0.08
	Olive oil	4.2 ± 0.2	66.0 ± 4.2	8.0 ± 0.7	18.9 ± 1.4	0.73 ± 0.12
Kidney	DBE	12.5 ± 1.1	44.7 ± 6.0	180 ± 20	94.3 ± 9.2	23.9 ± 1.1
	Olive oil	12.1 ± 0.9	47.4 ± 5.5	198 ± 18	99.4 ± 9.3	21.8 ± 0.6

[a] *U* is defined as 1 μmole NADPH oxidised/min.
[b] denotes the amount of ^{14}C amino acid incorporated during the reaction.
[c] *U* is defined at 1 μmole *p*-nitroaniline produced/min.
* Significantly different from control values, using Mann–Whitney *U*-test; $P < 0.01$.

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