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Turnover of hepatic glutathione after acute lindane intoxication

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SUMMARY

The administration of lindane (60 mg/kg) to fed rats diminished the content of hepatic glutathione (GSH) 4 h after treatment, which was recovered at 24 h. At these experimental times, the activities of glutathione peroxidase, glutathione reductase, glutathione-*S*-transferases and γ-glutamyltransferase in the liver of lindane-treated rats and control animals were comparable. Liver GSH turnover, measured after a pulse of [^{35}S]cysteine, was enhanced by 69% ($P < 0.05$) in lindane-treated rats 24 h after intoxication compared to controls, with a 63% ($P < 0.05$) increase in the estimated rate of GSH synthesis. It is concluded that lindane enhances GSH synthesis in rat liver 24 h after treatment as a consequence of the decrement in its content observed at early times of intoxication (4 h), thus allowing the recovery of the normal level of hepatic GSH.

INTRODUCTION

The development of an oxidative stress condition in the liver by acute lindane intoxication has been proposed as a possible hepatotoxic mechanism of the insecticide

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[1]. This condition is characterized by an enhancement in the rate of superoxide radical generation and of lipid peroxidation indicators, together with an alteration in the antioxidant defense mechanisms of the liver [2]. Among them, lindane induces a diminution in the hepatic content of glutathione (GSH) [3] as well as in the efflux of GSH across canalicular [3] and sinusoidal [4] membranes, exerted at early times after intoxication (4 h). In view of the fact that, under normal steady-state conditions, the hepatic GSH pool is maintained by continuous utilization and synthesis of the thiol [5], the study of the influence of lindane on the activity of the enzymes related to GSH utilization and on the turnover of hepatic GSH was undertaken, in order to get an insight into the recovery of the content of the tripeptide observed at later times of intoxication (24 h) [3].

MATERIALS AND METHODS

Fed male Wistar rats weighing 180–200 g received either a single i.p. dose of 60 mg/kg of body weight prepared in corn oil, or equivalent volumes of corn oil (controls) at 09:00 h, and were studied at different times after treatment for up to 24 h, or at 24 h in the turnover experiments. Total GSH equivalents (GSH + 2GSSG) [6] and enzyme activities were determined in livers previously perfused in situ with 200 ml of ice-cold 150 mM KCl containing 10 mM Tris (pH 7.4), chopped and homogenized (20% w/v) in the same buffer. Glutathione peroxidase activity [7] was measured in supernatants of liver homogenates centrifuged at $500 \times g$ for 5 min at 4°C. Glutathione reductase [8] and glutathione-*S*-transferases [9] (using either 1-chloro-2,4-dinitrobenzene (aryl substrate) or 1,2-epoxy-3-(*p*-nitrophenoxy) propane (epoxide substrate)) were determined in supernatants of liver homogenates centrifuged at $30\,000 \times g$ for 60 min at 4°C. γ-Glutamyltransferase activity was determined either in whole liver homogenates [10] or in the isolated perfused rat liver [11]. The latter determination was performed by infusing Krebs-bicarbonate buffer containing 0.08 to 0.7 mM of the synthetic substrate γ-glutamyl-*p*-nitroanilide at a fixed concentration of the synthetic acceptor glycylglycine (8 mM), gassed with 95% O_2/5% CO_2 gave pH 7.4, at a rate of 3–4 ml/g liver/min, at 36°C [11]. Formation of *p*-nitroaniline was measured spectrophotometrically at 405 nm [10] in caval perfusate aliquots taken every minute. One unit of enzyme activity corresponds to 1 μmol of product produced per min at 25°C. Protein was determined by the biuret method [12].

Hepatic GSH turnover was determined after a pulse of 200 μCi of L-[^{35}S]cysteine/kg body weight i.p. (1 Ci/μmol; Amersham International plc, Buckinghamshire) in a 0.9% w/v NaCl solution containing 10 mM dithiothreitol [13]. Control (n = 12) and lindane-treated (n = 14) rats were killed after 1, 1.67, 2, 2.33, and 3 h, and the livers were homogenized (10% w/v) in 0.5 M $HClO_4$ containing 5 mM EDTA, and centrifuged at $2000 \times g$ for 15 min at 4°C. The supernatants obtained were used to determine total radioactivity in [^{35}S]GSH and its specific radioactivity, after separation of GSH by HPLC in a C_{18}, 10 μ Bondapak-RP column, using 0.05% w/v H_3PO_4 as the eluting solvent, at a flow rate of 0.7 ml/min. GSH in the column effluent was meas-

ured spectrophotometrically [6], and its radioactivity was quantified by liquid scintillation counting.

All reagents used were obtained from Sigma Chemical Co. (St. Louis, MO). Values shown are means ± SE for the number of animals indicated. The statistical significance of differences between control and lindane-treated animals was determined by Student's test for unpaired results. The rate of GSH turnover in control rats (three separate trials comprising 12 animals) and in lindane-treated rats (four separate trials comprising 14 animals) was calculated from plots of the declining specific radioactivity of [^{35}S]GSH vs. time by least-square linear regression analysis. The significance of the difference in turnover rates was assessed by one-way analysis of variance for unequal size groups, using the random model.

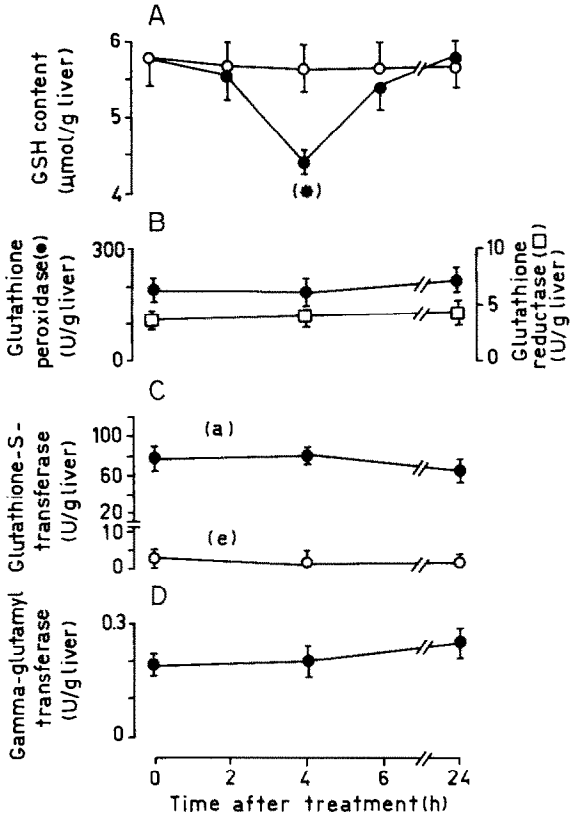

Fig. 1. Time-course of the changes in hepatic glutathione content and in the activity of enzymes involved in glutathione utilization in fed control and lindane-treated (60 mg/kg) rats. (A) Total GSH equivalents (GSH + 2GSSG) in the liver of control (○) and lindane-treated (●) rats; $P < 0.02$; (B) activity of glutathione peroxidase and glutathione reductase; (C) activity of glutathione-*S*-transferase, using aryl (a) and epoxide (e) substrates; (D) activity of γ-glutamyltransferase in whole liver homogenates. Values shown correspond to the means ± SE for 5 to 14 rats per experimental time, with control values shown at time zero.

RESULTS

Administration of lindane to fed rats elicited a significant diminution in the content of hepatic GSH (24%; $P < 0.02$) 4 h after treatment, which returned to control values at 6–24 h (Fig. 1A). Concomitantly, no significant changes were observed in the activity of either the glutathione peroxidase-glutathione reductase couple (Fig. 1B), glutathione-*S*-transferase (Fig. 1C), or γ-glutamyltransferase (Fig. 1D) in the liver of lindane-treated rats, 4 and 24 h after treatment, compared to control values (time zero), expressed as U/g liver (Fig. 1) or as U/mg protein (data not shown). The lack of effect of lindane on total γ-glutamyltransferase activity observed in whole liver homogenates (Fig. 1D) is in agreement with data obtained in the isolated perfused rat liver, which allows the evaluation of the basolateral activity of the enzyme [11] (apparent V_{max} values calculated from Lineweaver-Burk plots using linear regression analysis: controls, 0.049 U/g liver; lindane-treated rats 24 h after intoxication, 0.048).

The study of the influence of lindane on the turnover of hepatic GSH revealed a significant 69% increase in the first-order rate constant of [^{35}S]GSH disappearance from the liver, 24 h after treatment (Fig. 2). During the turnover studies, the hepatic GSH levels in control and lindane-treated rats were comparable at the different times studied, indicating steady-state conditions for the hepatic GSH pool in both experimental groups (Fig. 2, inset). In this situation, the amount of GSH synthesized per unit time can be estimated from the first-order rate constant of [^{35}S]GSH disappear-

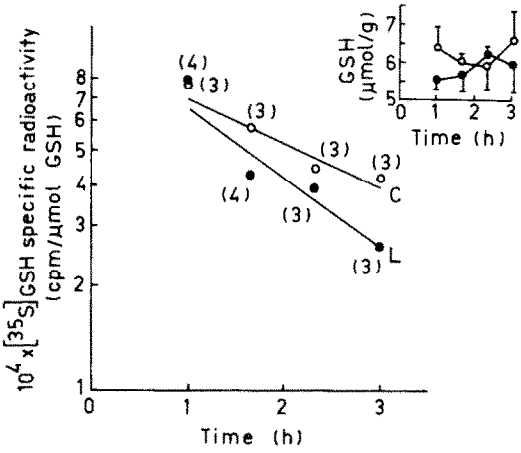

Fig. 2. Effect of lindane intoxication (60 mg/kg) on the rate of disappearance of [^{35}S]glutathione (GSH) from rat liver, 24 h after treatment. Numbers in parentheses adjacent to points correspond to the number of rats used, both for control (C) and lindane-treated (L) rats. The decay of [^{35}S]GSH followed a first-order process both in control ($r = -0.95$) and lindane-treated ($r = -0.95$) rats, with first-order rate constants of 0.122 ± 0.09 ($n = 3$) h^{-1} and 0.206 ± 0.008 ($n = 4$) h^{-1} ($P < 0.05$), respectively. Inset: liver total GSH content at different times after the pulse of [^{35}S]cysteine (time zero); values in each experimental group at the different, times studied were not significantly different, with mean values of 6.02 ± 0.37 ($n = 12$) μmol GSH/g liver and 5.80 ± 0.22 ($n = 14$) (not significant) in control and lindane-treated rats, respectively.

ance and the size of the glutathione pool [14], giving values of 12.20 ± 1.36 (n = 3) nmol/g liver/min in control rats and 19.91 ± 1.36 (n = 4) in lindane-treated animals (63% increase; $P < 0.05$; from Fig. 2).

DISCUSSION

Acute lindane administration in the rat elicited a significant diminution in the content of hepatic GSH, 4 h after intoxication. Since at this experimental time, the canalicular [3] and sinusoidal [4] efflux of GSH, components accounting for 90–95% of the rate of hepatic GSH utilization [5], are decreased by lindane, the depletion of hepatic GSH observed could be related to utilization of the tripeptide for conjugation with lindane-derived electrophilic metabolites [15] and/or in peroxide metabolism coupled to the onset of lipid peroxidation [2]. These GSH-dependent processes seem to adequately operate, as the activity of glutathione-*S*-transferases and that of the glutathione peroxidase-glutathione reductase couple were not modified by the treatment. Repletion of hepatic GSH occurred 24 h after lindane treatment, together with a marked increase in the turnover of the tripeptide, implying an enhancement in the rate of GSH synthesis by the liver. This adaptive response could be triggered by the low levels of GSH that prevail at 4 h after lindane treatment, and would allow the recovery of the content of hepatic GSH (Fig. 1A) and its efflux into bile [3] and blood [4] observed at 24 h, in conditions of a sustained GSH utilization in conjugation and/or oxidation processes.

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