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PROSTACYCLIN ELEVATION FOLLOWING GLUTATHIONE DEPLETION *IN VIVO*

POSSIBLE THRESHOLD DEPENDENCY IN LIVER AND LUNG

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Abstract—The major objective of this study was to determine if a threshold level of glutathione (GSH) depletion is required to elevate plasma prostacyclin (6-ketoPGF$_{1\alpha}$) in male Sprague–Dawley rats. Rats were treated i.p. with various doses of phorone, diethyl maleate (DEM), or GSH with and without DEM. Similar maximal depletions of hepatic GSH (to 10% of control) and renal GSH (to 50% of control) were observed with DEM and phorone, but lung GSH was depleted maximally by only 30% with phorone compared with a 70% depletion by DEM. Changes in lung GSH, but not kidney GSH, were closely correlated with changes in hepatic GSH. 6-KetoPGF$_{1\alpha}$ levels in the lung were 10- to 30-fold higher than in kidney or liver, and there was a stronger correlation between lung and plasma 6-ketoPGF$_{1\alpha}$ than with the other two tissues. The increase in lung 6-ketoPGF$_{1\alpha}$ following GSH depletion did not appear to be due to a shift in prostaglandin metabolite synthesis since reciprocal changes in PGE_2 were not observed; lung PGE_2 levels were largely unaffected by DEM or phorone. Both DEM and phorone elevated plasma 6-ketoPGF$_{1\alpha}$ but the magnitude of increase for DEM (5- to 6-fold) was much greater than the 2-fold increase for phorone. The increase in plasma 6-ketoPGF$_{1\alpha}$ by 1.0 mL DEM/kg was attenuated by simultaneous administration of 2 mmol GSH/kg. The results indicate that the lung may be responsible for increases in plasma 6-ketoPGF$_{1\alpha}$ following GSH depletion and that a critical level of GSH depletion in the liver and/or lung may be necessary to elevate plasma 6-ketoPGF$_{1\alpha}$ levels.

Reduced glutathione (GSH†) appears to play an important modulatory role in the synthesis of arachidonic acid metabolites such as prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2). The synthesis of these compounds depends on the availability of arachidonate as well as the activities of synthetic enzymes. Prostaglandin H synthase contains both endoperoxidase and cyclooxygenase components and catalyzes the conversion of arachidonate to PGH_2. Inhibition of cyclooxygenase activity by GSH in the presence of excess GSH peroxidase was first demonstrated by Smith and Lands [1]. This effect has been attributed to GSH peroxidase removal of hydroperoxides that activate cyclooxygenase [2, 3]. Although PGI_2 synthase has no specific requirement for GSH, low levels of GSH may be needed for optimal activity to prevent inactivation by lipid peroxides [4]. In contrast, GSH is a cofactor for enzymatic conversion of PGH_2 to PGE_2 by PGE_2 isomerase, but there is evidence that PGE_2 is produced non-enzymatically as well [5].

Relatively few studies have determined effects of GSH alterations on prostaglandin production in intact cells, and even fewer studies have investigated effects of GSH alterations *in vivo*. In renal medullary homogenates, elevations in GSH were associated with an increase in PGE_2 synthesis while the synthesis of all other prostanoids was suppressed [6]. Rouzer *et al.* [7] reported that GSH depletion to 3% in macrophages by buthionine sulfoximine (BSO) produces a 90–95% decrease in PGE_2 synthesis with an equal and concomitant increase in 6-ketoPGF$_{1\alpha}$ (the stable metabolite of PGI_2). LaSierra *et al.* [8] demonstrated an inverse relationship between BSO-mediated hepatic GSH depletion and increases in 6-ketoPGF$_{1\alpha}$ synthesis in rabbit aortic rings *ex vivo*. Using several different GSH-depleting agents, Buckley *et al.* [9] also demonstrated an inverse relationship between GSH content and 6-ketoPGF$_{1\alpha}$ synthesis in aortic endothelial cells and concluded that cellular GSH levels may influence vascular function by regulating endothelial prostaglandin synthesis. Thus, alterations in GSH that occur during oxidative or xenobiotic insult may produce blood flow changes by alterations in vasoactive prostanoid

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† Abbreviations: BSO, buthionine sulfoximine; DEM, diethyl maleate; GSH, glutathione; PG, prostaglandin; PGI_2, prostacyclin (6-ketoPGF$_{1\alpha}$); and PGE_2 prostaglandin E_2.

synthesis as has been reported [10–13]. However, unlike the findings by LaSierra *et al.* [8] and Buckley *et al.* [9] in which inverse relationships between GSH and 6-ketoPGF$_{1\alpha}$ were observed *in vitro*, it was hypothesized that a threshold level of GSH depletion may be required to elevate 6-ketoPGF$_{1\alpha}$ synthesis *in vivo* [10, 14].

The liver, kidney and lungs play major roles in GSH metabolism and detoxification of numerous xenobiotics [15–18]. The liver is vital to GSH homeostasis since it provides tissues with GSH by interorgan transport followed by uptake from the blood by organs such as the kidney and lungs through the action of γ-glutamyl transpeptidase [19]. Prostaglandin-induced alterations in hepatic [10, 11] or intestinal [12] blood flow could therefore be very important in GSH homeostasis during oxidative stress. For these reasons, the major objective of this study was to utilize GSH-depleting agents in dose–response experiments to determine relationships between tissue GSH and 6-ketoPGF$_{1\alpha}$ in plasma, lungs, liver and kidney. A secondary objective was to determine PGE$_2$ levels in lung tissue to ascertain if high levels of 6-ketoPGF$_{1\alpha}$ following GSH depletion [14] could be attributed to a shift in prostaglandin metabolite formation.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, MA). The rats were maintained three to a cage on a 12-hr light schedule (8:00 a.m. to 8:00 p.m.) and provided food and water *ad lib*. The rats weighed 410 ± 22 g at the time the experiments were conducted.

Chemicals

Phorone was obtained from the Aldrich Chemical Co., Milwaukee, WI. All other chemicals were obtained from the Sigma Chemical Co., St. Louis, MO.

Experimental protocol

Experiments were carefully timed so that all samples were obtained between 10:00 a.m. and noon each day to minimize differences that might have occurred due to diurnal fluctuations in GSH [20].

Experiment 1: The objective of Expt. 1 was to determine the effect of several doses of phorone on tissue GSH, tissue and plasma 6-ketoPGF$_{1\alpha}$, and lung PGE$_2$. Phorone was distilled to remove impurities. Thirty male rats were randomly assigned to 0 (control), 50, 100, 150, 200 or 250 mg phorone/kg (N = 5/treatment). Each dose was mixed with corn oil and the total amount (0.6 mL/kg) injected i.p. Blood samples and tissues were obtained as previously described [14]. One hour after treatment, the rats were anesthetized with ketamine hydrochloride (60 mg/kg) and xylazine (6 mg/kg). Under anesthesia (5 min later), a 4-mL blood sample (by heart puncture) was withdrawn into a syringe containing an ice-cold aspirin–heparin–saline solution (100 U heparin/mL saline) for a final concentration of 0.48 mmol aspirin. The blood was centrifuged at 5° and 5000 *g* for 2.5 min. Plasma was frozen immediately in liquid nitrogen and stored at −80°. Portions of the lung, liver and both kidneys were then removed, frozen in liquid nitrogen, and stored at −80°.

Experiment 2: In Expt. 2, 45 male rats were randomly assigned to one of nine treatments (N = 5/treatment). These included 0 (control, C) 0.2, 0.4, 0.6, 0.8 and 1.0 mL DEM/kg (or 0 to 6 mM DEM/kg body wt). Each DEM treatment was mixed with corn oil for a total volume of 2 mL/kg. An additional group was treated with distilled DEM (D-DEM, 1.0 mL/kg mixed 1:1 with corn oil) to determine if removal of impurities by distillation could alter GSH depletion or prostanoid production. All rats in the treatments described above also were injected (i.p.) with 1 mL saline/kg to control for saline injections in remaining treatment groups. Since the highest dose of DEM increases plasma 6-ketoPGF$_{1\alpha}$ [10, 14, 21], one group (DEM/GSH) received 2 mmol GSH/kg (dissolved in saline, total injection volume of 1 mL/kg) simultaneously with the injection of the 1.0 mL DEM/kg dose to determine if exogenously administered GSH would attenuate increases in 6-ketoPGF$_{1\alpha}$ synthesis. A GSH control group (C + GSH) received 2 mmol GSH/kg (in saline) with 2 mL corn oil/kg. One hour after i.p. injection of each treatment solution, the rats were anesthetized. Blood and tissue samples were obtained as described in Expt. 1 above.

Determination of tissue and plasma 6-ketoPGF$_{1\alpha}$ and PGE$_2$

Tissues were homogenized in an aspirin solution to a final concentration of 0.48 mmol [22] and centrifuged for 20 min at 2500 *g*. Tissue supernatant and plasma levels of 6-ketoPGF$_{1\alpha}$ and/or PGE$_2$ were determined by radioimmunoassay with a sequential double antibody procedure equilibrated at 4° [23–25] using tritiated metabolites (Dupont Chemical Co., MA). The primary antibodies have been examined extensively and cross-reactivities with other prostaglandins have been reported previously [10, 25]. Prostaglandin values were calculated using specific radioimmunoassay software (SECURIA, Packard Instrument Co., Downers Grove, IL).

Glutathione analysis

Tissue concentrations of reduced (GSH) and oxidized (GSSG) glutathione were determined by HPLC according to Fariss and Reed [26] as previously described [10, 14]. Since GSSG levels in both experiments were less than 2% of GSH in all three tissues, and 2–4 observations in each tissue and treatment had GSSG levels below the limits of detection (0.03 μmol/g), only GSH levels are reported.

Statistics

Data are presented as means ± SEM. Analysis of variance and linear regression analysis were carried out using the general linear model procedure of SAS [27]. Regression equations and differences in mean values were considered significant at $P < 0.05$.

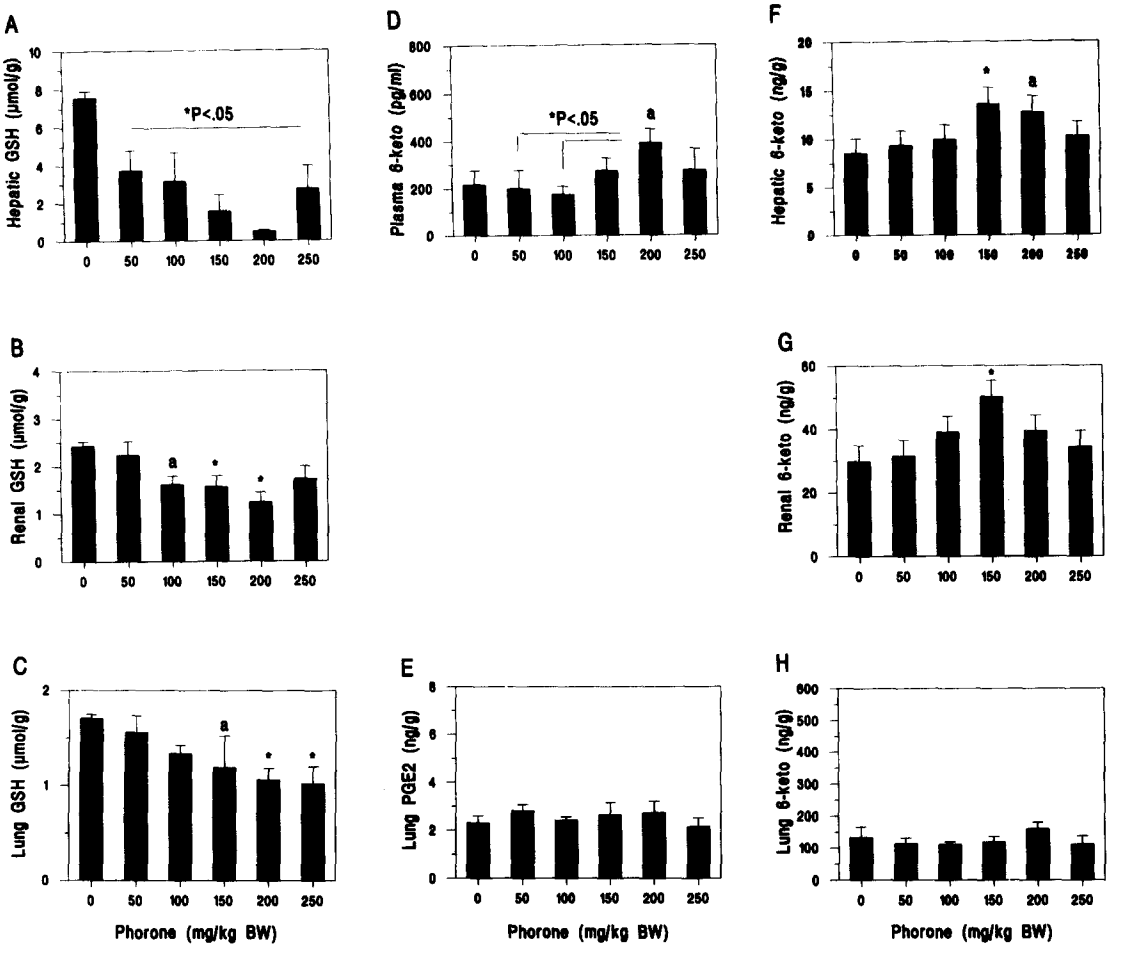

Fig. 1. (A) Hepatic, (B) renal, and (C) lung tissue glutathione (GSH), (D) plasma 6-ketoPGF$_{1\alpha}$ (6-keto), (E) lung PGE_2 and (F) hepatic, (G) renal, and (H) lung 6-ketoPGF$_{1\alpha}$ (6-keto) concentrations in male rats 1 hr after i.p. injection of phorone (0–250 mg/kg body wt) in Expt. 1. The bars represent the means ± SEM for 4–5 observations. Key: (*) treatment means were significantly different from control (0 mg/kg body wt) at $P < 0.05$; and (a) treatment means were different from control (0 mg/kg body wt) at $P < 0.1$.

RESULTS

The effects of phorone on tissue GSH and prostanoid concentrations are presented in Fig. 1. Phorone depleted hepatic GSH ($P < 0.05$, Fig. 1A), and maximal hepatic GSH depletion (to approximately 7% of control) occurred in the 200 mg/kg treatment group. Two rats in the 250 mg/kg treatment inexplicably exhibited hepatic GSH values that were 70–80% of control (mean hepatic GSH for these two rats was 6.02 µmol/g), whereas mean hepatic GSH in the remaining three rats was 0.82 µmol/g, or approximately 10% of control. (Mean plasma 6-ketoPGF$_{1\alpha}$ values for these high and low hepatic GSH rats in this treatment group were 175 and 341 pg/mL, respectively; see Fig. 1D.) The profile of renal GSH depletion by phorone was similar to that of the liver with the 150 and 200 mg/kg treatment groups exhibiting the lowest renal GSH levels (Fig. 1B). The slightly higher renal GSH values in the 250 mg/kg treatment group were not different ($P > 0.1$) from control. In contrast, lung GSH was depleted by the 150 mg/kg dose ($P < 0.1$), and the 200 and 250 mg/kg doses ($P < 0.05$) in comparison with controls.

Plasma 6-ketoPGF$_{1\alpha}$ concentrations were increased approximately 2-fold by the 200 mg/kg treatment in comparison with the control ($P < 0.07$), 50 and 100 mg/kg treatments ($P < 0.05$, Fig. 1D). Whereas phorone had no effect on lung PGE_2 (Fig. 1E) or lung 6-ketoPGF$_{1\alpha}$ (Fig. 1H), hepatic and renal 6-ketoPGF$_{1\alpha}$ levels were elevated ($P < 0.05$) by the 150 mg/kg treatment in comparison with the controls (Fig. 1, F and G, respectively).

The effects of DEM and/or GSH are presented in Fig. 2. The injection of 2 mmol GSH/kg (C + GSH) had no effect on hepatic GSH (Fig. 2A) but increased ($P < 0.05$) GSH levels in both the kidney (Fig. 2B) and lung (Fig. 2C). Presumably, elevations in lung and renal, but not liver GSH, were due to higher γ-glutamyl transpeptidase activity

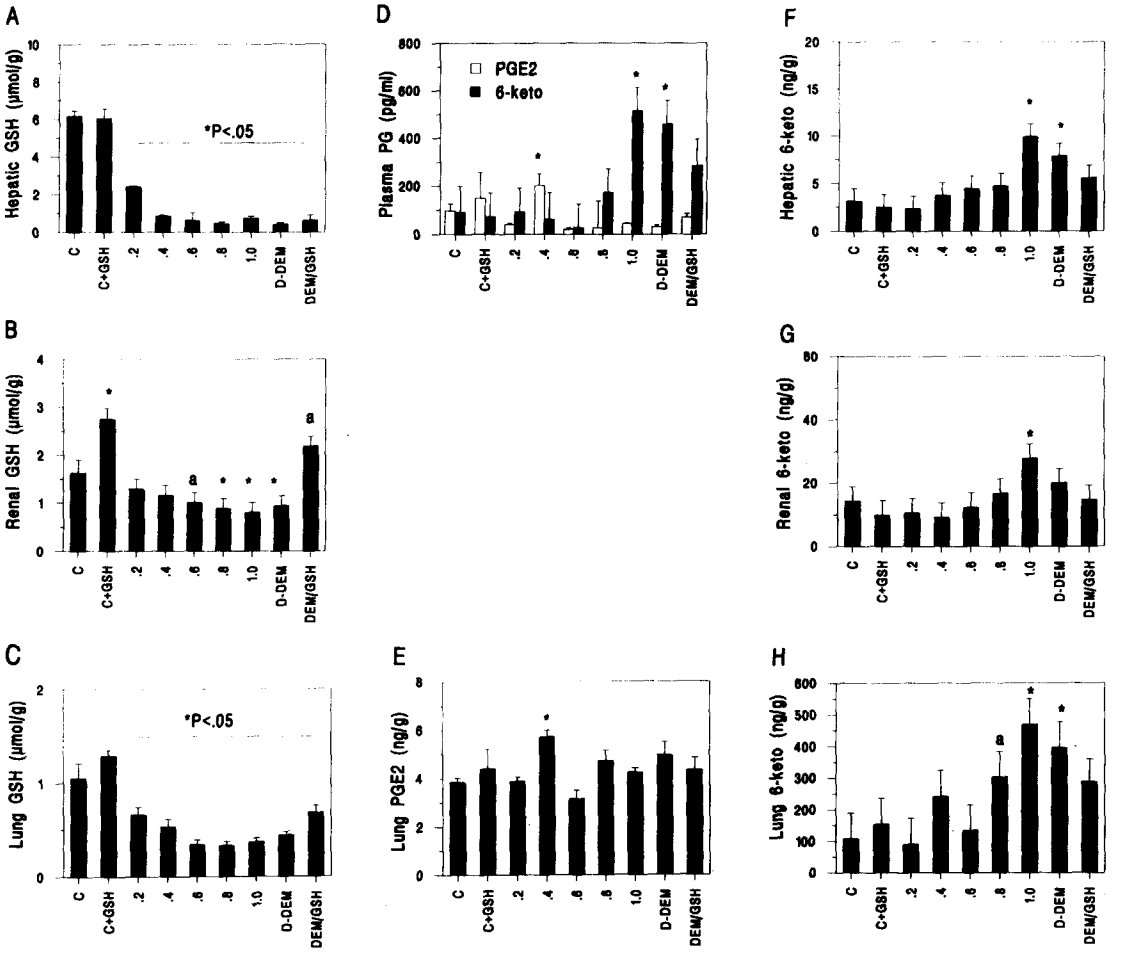

Fig. 2. (A) Hepatic, (B) renal, and (C) lung tissue glutathione (GSH), (D) plasma 6-ketoPGF$_{1\alpha}$ (6-keto) and PGE$_2$, (E) lung PGE$_2$, and (F) hepatic, (G) renal, and (H) lung 6-ketoPGF$_{1\alpha}$ (6-keto) concentrations in male rats 1 hr after treatment in Expt. 2. Treatments included i.p. injections of diethyl maleate (DEM) of 0 (control, C), 0.2 to 1.0 mL/kg body wt mixed with corn oil (total injection, 2 mL/kg body wt), 2 mL corn oil with 2 mmol GSH/kg body wt (C + GSH), 1 mL distilled DEM l/kg body wt (v/v corn oil) (D-DEM), and 1 mL DEM/kg body wt (v/v corn oil) with 2 mmol GSH/kg body wt (DEM/GSH). The bars represent the means ± SEM for 4–5 observations. Key: (*) treatment means were significantly different from control at $P < 0.05$; and (a) treatment means were different from control at $P < 0.1$.

in these organs in comparison with activity levels in the liver [19]. DEM (0.2 to 1.0 mL/kg) lowered ($P < 0.05$) hepatic and lung GSH in comparison with controls, whereas renal GSH was decreased ($P < 0.05$) with 0.8 and 1.0 mL/kg only. Distillation of DEM had no effect on GSH depletion since there were no differences in GSH depletion between the 1.0 mL DEM and 1.0 mL D-DEM/kg treatments. Simultaneous injection of 2 mmol GSH with 1.0 mL DEM/kg (DEM/GSH) had no effect on hepatic GSH (Fig. 2A) but attenuated ($P < 0.1$) the magnitude of lung GSH depletion compared with rats receiving 1.0 mL DEM/kg (Fig. 2C). Renal GSH levels in the DEM/GSH group were elevated ($P < 0.1$) compared with controls (Fig. 2B).

Plasma PGE$_2$ and 6-ketoPGF$_{1\alpha}$ concentrations from Expt. 2 are presented in Fig. 2D. Plasma 6-ketoPGF$_{1\alpha}$ levels were higher ($P < 0.05$) than controls following 1.0 mL DEM or D-DEM/kg, and this increase was attenuated by 2 mmol of GSH (DEM/GSH). Plasma PGE$_2$ was higher ($P < 0.05$) in rats receiving 0.4 mL DEM/kg compared with controls and 0.2, 0.6, 1.0 mL DEM/kg and D-DEM treatments. Similarly, lung PGE$_2$ levels were higher ($P < 0.05$) after 0.4 mL DEM/kg compared with 0, 0.6 and 1.0 mL DEM/kg treatments (Fig. 2E). The 1.0 mL DEM/kg treatment elevated ($P < 0.05$) 6-ketoPGF$_{1\alpha}$ levels in all three tissues (Fig. 2, F–H) and 0.8 mL DEM/kg increased lung 6-ketoPGF$_{1\alpha}$ slightly ($P < 0.1$) in comparison with controls. The levels of 6-ketoPGF$_{1\alpha}$ were also elevated ($P < 0.05$) by the D-DEM treatment in hepatic and lung tissue (Fig. 2, F and H) but not in kidney (Fig. 2G).

A plot of lung 6-ketoPGF$_{1\alpha}$ and PGE$_2$ con-

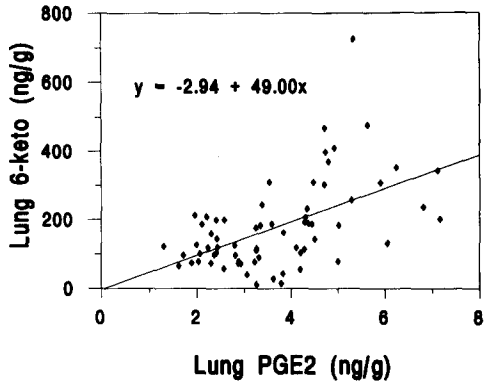

Fig. 3. Relationships between lung PGE_2 and 6-ketoPGF$_{1\alpha}$ (6-keto) concentrations of tissues obtained from rats in Expts. 1 and 2. The regression equation shown was significant at $P < 0.05$ ($r^2 = 0.29$).

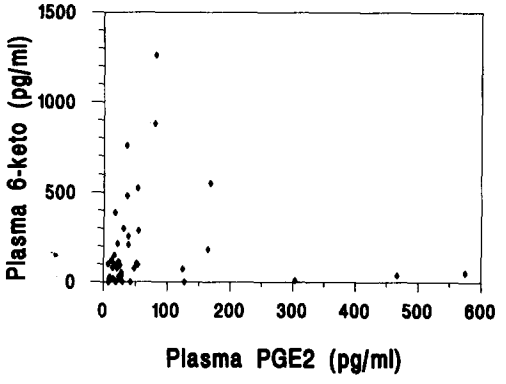

Fig. 4. Relationships between plasma PGE_2 and 6-ketoPGF$_{1\alpha}$ (6-keto) concentrations obtained from rats in Expt. 2.

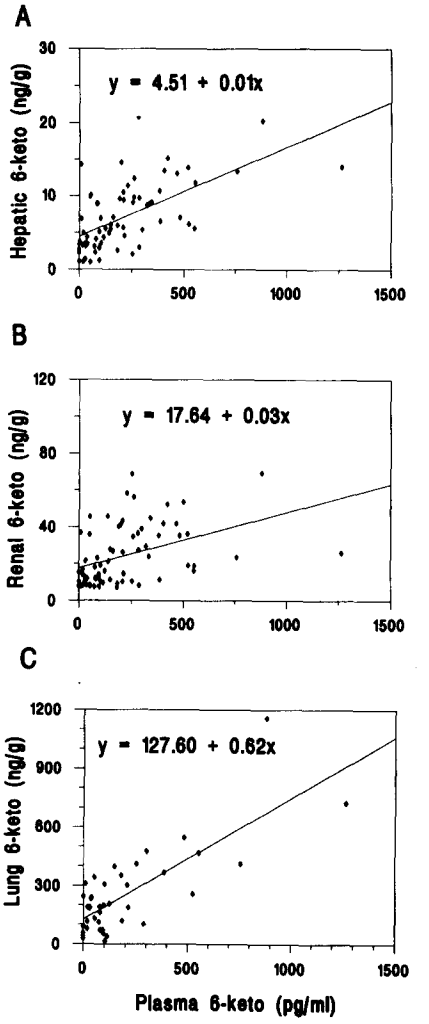

Fig. 5. Relationships between plasma 6-ketoPGF$_{1\alpha}$ (6-keto) and 6-ketoPGF$_{1\alpha}$ (6-keto) concentrations in (A) hepatic, (B) renal, and (C) lung tissues obtained from rats in Expts. 1 and 2. The regression equations shown were significant at $P < 0.05$ in hepatic tissue ($r^2 = 0.37$) and lung tissue ($r^2 = 0.60$) and at $P < 0.1$ in renal tissue ($r^2 = 0.18$).

centrations in rats from both experiments (Fig. 3) indicates that a shift in prostaglandin metabolite synthesis did not occur in lung tissue following GSH depletion. However, the graph in Fig. 4 of data from Expt. 2 seems to indicate a different relationship between plasma PGE_2 and 6-ketoPGF$_{1\alpha}$, i.e. there is indication of an inverse relationship between concentrations of these prostaglandins in plasma especially when either was very high.

Relationships between tissue and plasma 6-ketoPGF$_{1\alpha}$ concentrations are depicted in Fig. 5. The regression equations and correlation coefficients were significant at $P < 0.05$ for hepatic (Fig. 5A) and lung (Fig. 5C) tissues and at $P < 0.1$ for renal tissue (Fig. 5B). It is apparent that a stronger correlation exists between lung and plasma 6-ketoPGF$_{1\alpha}$ levels than between plasma and tissue 6-ketoPGF$_{1\alpha}$ levels in either the liver or the kidney.

Relationships between relative changes in hepatic GSH with renal and lung GSH expressed as a percent of control are shown in Fig. 6, A and B, respectively. Relative changes rather than actual values were used due to differences in control tissue GSH between the two experiments (see Figs. 1 and 2). The data in Figs. 6 and 7 were determined on mean values in the present study and from one in which rats were treated with DEM or BSO [14]. While there was no significant correlation between hepatic and renal GSH (Fig. 6A), the regression between percent changes in lung and hepatic GSH (Fig. 6B) was significant ($P < 0.05$, $r^2 = 0.76$). Thus, changes in lung GSH were more closely linked with changes in hepatic GSH than were the changes in renal GSH.

Relative changes in plasma 6-ketoPGF$_{1\alpha}$ and tissue GSH are shown in Fig. 7. It appears that a threshold level of hepatic GSH depletion of 20% of control was necessary to elevate plasma 6-ketoPGF$_{1\alpha}$ concentrations slightly, but a large increase in plasma

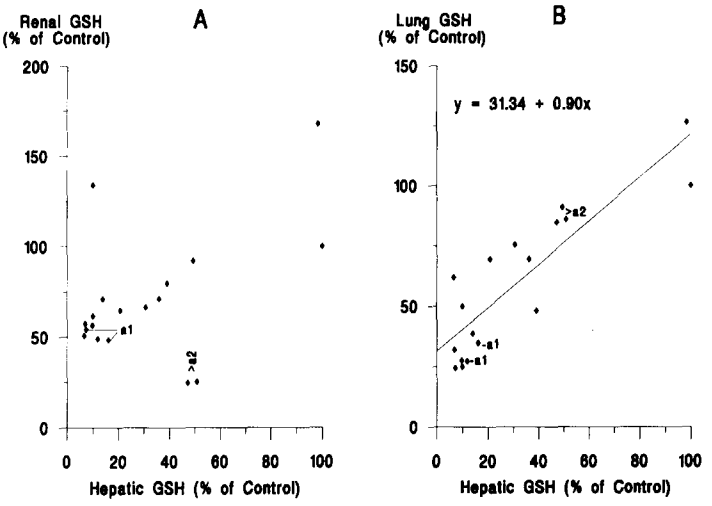

Fig. 6. Relationships between changes in (A) renal and hepatic GSH, and (B) lung and hepatic GSH expressed as a percent of control. Control tissue GSH values are provided in Figs. 1 and 2. Each data point represents the mean value of 4–5 observations obtained in Expts. 1 and 2. The points associated with a1 and a2 were calculated from data by Maynard *et al.* [14] which utilized DEM and buthiomine sulfoximine, respectively, to deplete GSH. The regression equation shown in B was significant at $P < 0.05$ ($r^2 = 0.76$).

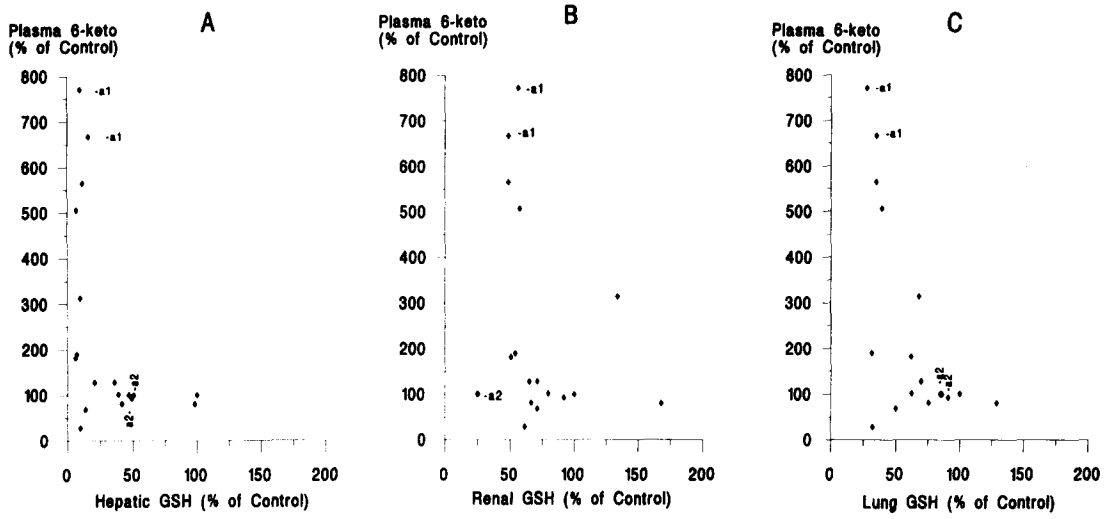

Fig. 7. Relationships between changes in (A) hepatic, (B) renal, and (C) lung GSH concentrations and plasma 6-keto$PGF_{1\alpha}$ concentrations expressed as a percent of control. Control tissue GSH values and plasma 6-keto$PGF_{1\alpha}$values are provided in Figs. 1 and 2. Each data point represents the mean value of 4–5 observations obtained in Expts. 1 and 2. The points associated with a1 and a2 were calculated from data by Maynard *et al.* [14] which utilized DEM and buthiomine sulfoximine, respectively, to deplete GSH.

6-keto$PGF_{1\alpha}$ was observed when hepatic GSH levels were less than 20% (Fig. 7A). Relationships between renal GSH and plasma 6-keto$PGF_{1\alpha}$ are presented in Fig. 7B. It is doubtful that alterations in kidney GSH content had much impact on plasma 6-keto$PGF_{1\alpha}$ levels (see Figs. 5 and 6). When lung GSH dropped to less than 70% of controls, an increase in plasma 6-keto$PGF_{1\alpha}$ levels was observed (Fig. 7C). Using the regression equation from Fig. 6B, a 20% depletion of hepatic GSH would correspond to approximately a 50% depletion in lung GSH. It can be seen in Fig. 7C that a 50% depletion would likely be associated with an increase in plasma 6-keto$PGF_{1\alpha}$.

DISCUSSION

Tissue prostanoid levels in this study reflect both endogenous concentrations as well as that contributed by blood remaining in the tissue at freezing. Lung 6-keto$PGF_{1\alpha}$ levels were 10- to 30-fold higher than in kidney or liver and there was a stronger correlation between lung and plasma 6-keto$PGF_{1\alpha}$ than with the other two tissues. Consequently, the lung may be primarily responsible for the increase in plasma 6-keto$PGF_{1\alpha}$ following acute GSH depletion observed in this and previous [10, 14, 21] studies. The increase in lung 6-keto$PGF_{1\alpha}$ did not appear to be due to a shift in prostaglandin metabolite synthesis, such as was demonstrated in macrophages [7], since reciprocal changes in PGE_2 were not observed.

An explanation for the observed increase in plasma 6-keto$PGF_{1\alpha}$ with no change in plasma PGE_2 concentrations (Fig. 2D) may be attributed to antagonistic processes. GSH depletion by DEM may have raised peroxide tone to levels sufficient to stimulate cyclooxygenase [3], while enough GSH remained to maintain PGI_2 synthase activity [4]. Increased cyclooxygenase activity could also stimulate synthesis of other prostanoids such as was observed for thromboxane [10]. However, with PGE_2, this potential elevation due to increased cyclooxygenase activity would be negated if GSH levels were low enough to attenuate PGE_2 isomerase activity [28]. An apparent K_m of PGE_2 isomerase activity of approximately 0.1 mM can be calculated from data presented by Ogino *et al.* [28]. The data in Fig. 2A in which hepatic GSH levels were lowered to approximately 0.2 mM might suggest that GSH depletion approached this K_m, thus negating the influence exerted by increased peroxide tone. The obvious shift in prostaglandin metabolite formation noted by Rouzer *et al.* [7] may have occurred since GSH levels were lowered below the K_m for PGE_2 isomerase.

Both phorone and DEM rapidly deplete GSH by conjugation catalyzed by GSH-S-transferase [29]. Although similar magnitudes of hepatic and renal GSH depletion (to approximately 90 and 50% of control, respectively) were produced by both agents, lung GSH was depleted by 70% with DEM compared with only a 30% depletion with phorone. The reason for this difference in pulmonary GSH depletion by these agents is not apparent but is an important point in this report since the lungs have a vast amount of endothelium with considerable PGI_2 synthesizing capacity [30]. The elevation in plasma 6-keto$PGF_{1\alpha}$ was much greater for DEM (~550%) than for phorone (95%), indicating that the magnitude of lung GSH depletion may be critical for raising plasma 6-keto$PGF_{1\alpha}$ levels. The greater depletion of lung GSH by DEM may account in part for the higher plasma 6-keto$PGF_{1\alpha}$ levels in comparison with phorone-treated rats.

Buthionine sulfoximine elevates PGI_2 synthesis in intact cells [7–9] but has no effect on tissue or plasma 6-keto$PGF_{1\alpha}$ levels *in vivo* [14]. In the study by Maynard *et al.* [14], BSO depleted hepatic and renal GSH maximally to 34 and 25% of controls, respectively, but pulmonary GSH levels were decreased by only 15%. As in the present study, depletion of lung GSH to approximately 30% of control by DEM was associated with increased plasma 6-keto$PGF_{1\alpha}$ [14]. These data lend additional support to the concept that the magnitude of lung GSH depletion is important for elevating plasma 6-keto$PGF_{1\alpha}$. It should also be noted that in the *in vitro* studies, which demonstrated increased PGI_2 synthesis with BSO, endothelial GSH was depleted to 15% [9] and macrophage GSH was depleted to 3% of controls [7], values that are below the apparent critical level of GSH depletion required to elevate 6-keto$PGF_{1\alpha}$ levels *in vivo* (Fig. 7).

The concept of a critical level of GSH depletion as a key event in xenobiotic stress is not new. Several studies reported that deleterious increases in intracellular calcium homeostasis or lipid peroxidation do not occur prior to reaching a critical level of GSH depletion [31–33]. Lipid peroxidation, protein thiol depletion and increases in cellular calcium following GSH depletion are key events leading to cell death [31, 34]. Interestingly, Maellaro *et al.* [35] indicated that lipid peroxidation and liver damage in mice occur long after maximal GSH depletion: 1–2 hr with allyl alcohol, 9–12 hr with bromobenzene, and 14 hr with DEM. Thus, elevations in 6-keto$PGF_{1\alpha}$ following GSH depletion in this and earlier studies [10, 14, 21] might be considered as an early signal of toxicosis. A similar hypothesis was proposed recently by Kawada *et al.* [36] with respect to increases in PGI_2 synthesis during endotoxin-induced hepatic cell necrosis. Since PGI_2 is known to be cytoprotective (see, for example, Refs. 37–39), increased PGI_2 synthesis may serve in protecting cells during xenobiotic insult.

There is some controversy concerning the use of DEM as a GSH-depleting agent. While DEM has been criticized for having various side-effects [15], other reports indicated that DEM did not cause endothelial cell damage [9] or alter hepatic function [40, 41], nor was lipid peroxidation observed in liver or heart tissue following 2 hr of treatment of mice with 6 mmol DEM/kg [42]. Costa and Murphy [43] reported that DEM, but not phorone, inhibits protein synthesis. However, both agents decrease body temperature, whereas, BSO has no effect on body temperature [43]. Buckley *et al.* [9] noted that the increase in PGI_2 synthesis (approximately 250%) with DEM treatment of aortic endothelial cells was greater than with either BSO or 1-chlorodinitrobenzene, which increased PGI_2 synthesis by approximately 40 and 50%, respectively. Possibly, in addition to GSH depletion, DEM stimulates prostaglandin production, e.g. by acting as a calcium ionophore or by stimulating phospholipase A_2 activity. However, if DEM stimulates prostaglandin metabolism directly, one might predict that DEM would produce a dose-dependent increase in plasma 6-keto$PGF_{1\alpha}$. There is some indication of a dose-dependent increase in lung 6-keto$PGF_{1\alpha}$ (Fig. 2H) but not in plasma (Fig. 2D) in which 6-keto$PGF_{1\alpha}$ levels were depressed slightly by 0.2, 0.4 and 0.6 mL/kg DEM. The reason for the difference in the profile of 6-keto$PGF_{1\alpha}$ levels in lung tissue and plasma is not apparent since the lung has a limited capacity to metabolically inactivate PGI_2 [30].

An interesting observation was that GSH levels in tissues from controls in Expt. 2 that received 2 mL corn oil/kg were considerably lower than in controls in Expt. 1 in which rats were injected with 0.6 mL corn oil/kg. Hepatic, renal and lung GSH control values in Expt. 2 were approximately 85, 66 and 64%, respectively, of corresponding control tissue GSH values in Expt. 1. Lower tissue GSH levels were also observed in rats treated with higher amounts of corn oil [14]. The corn oil had been obtained from a commercial source shortly before the experiments were conducted and kept at 4°. Furthermore, animal treatment and tissue collection for both Expts. 1 and 2 were conducted on successive weeks, which should not have been enough time to cause any change in the oil. These observations indicate that higher amounts of corn oil may cause moderate depletions in tissue GSH.

In Expt. 2, i.p. administration of 2 mmol GSH/kg had no effect on hepatic GSH content but increased both lung and renal GSH. These findings concur with those of Griffith and Meister [19]. However, these results contrast with a recent report in which 1.67 mmol GSH/kg given i.v. to male rats increased both hepatic and renal GSH [44]. The reason for the differences between these studies is not apparent but may be related to route of administration (i.v. vs i.p.) or age, since body weights in the present study were nearly twice as high as in the study by Aebi and Lauterberg [44].

The results of this study provide evidence that lung GSH was more closely related to hepatic GSH status than was renal GSH. The lung appeared to be a principal source of 6-ketoPGF$_{1\alpha}$ in the plasma following GSH depletion, and a critical level of GSH depletion to approximately 20 and 70%, in the liver and lung, respectively, may be required before elevations in circulating levels of 6-ketoPGF$_{1\alpha}$ are observed. These findings suggest that xenobiotics that have a major effect on lung GSH content may be more likely to elevate PGI$_2$ synthesis than ones that predominantly affect hepatic GSH with little alteration of lung GSH.

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