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EFFECT OF GLUTATHIONE DEPLETION ON TISSUE AND PLASMA PROSTACYCLIN AND THROMBOXANE IN RATS

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Abstract—Experiments were designed to determine the effects of glutathione (GSH) depletion with L-buthionine sulfoximine (BSO) or diethyl maleate (DEM) on tissue and plasma prostacyclin (6-ketoPGF$_{1\alpha}$) and thromboxane (TxB_2) levels in male Sprague–Dawley rats. Despite depleting hepatic GSH to as much as 34% of control, BSO at various levels (0.4, 0.8 and 1.2 g/kg body wt) had no effect on hepatic, renal, pulmonary or cardiac tissue levels of 6-ketoPGF$_{1\alpha}$ and TxB_2 or circulating levels of 6-ketoPGF$_{1\alpha}$ in portal or arterial plasma. When rats were pretreated with 3-methylcholanthrene (3-MC) to induce cytochrome P450, BSO (0.8 g/kg body wt) also had no effect on tissue or plasma prostanoid levels with the exception of a slight, but significant, increase in hepatic 6-ketoPGF$_{1\alpha}$ in non-induced rats. In contrast, depletions of hepatic, renal and pulmonary tissue GSH by DEM (1 mL/kg body wt) to 12, 50 and 30% of control, respectively, were associated with elevations of 6-ketoPGF$_{1\alpha}$ in these tissues and in plasma obtained by right ventricular heart puncture. Pretreatment of rats with 3-MC had no significant effect on tissue GSH or prostanoid levels in controls or DEM-treated rats but plasma levels of 6-ketoPGF$_{1\alpha}$ were lower in comparison to non-induced rats. DEM with or without 3-MC pretreatment was associated with increased TxB_2 in renal tissue, whereas DEM elevated TxB_2 only in pulmonary tissue from non-induced rats. It appears that factors besides GSH depletion may be required to raise plasma and/or tissue 6-ketoPGF$_{1\alpha}$ levels *in vivo*.

Chemical manipulation of glutathione (GSH‡), an important endogenous antioxidant involved in detoxification and protection of cells from reactive oxygen species, has proven to be a useful tool in studying GSH metabolism [1, 2]. Diethyl maleate (DEM) depletes GSH in mammals to 10–30% of control between 45 and 60 min after administration [2–6] by conjugation followed by elimination of the DEM–GSH conjugate [7]. Buthionine sulfoximine (BSO) decreases GSH by inhibition of γ-glutamyl synthetase, the rate-limiting enzyme in GSH synthesis [2]. Although maximal GSH depletion by BSO is attained over a longer time period (2–7 hr) [2, 8, 9] in comparison to DEM, BSO may be a preferred GSH-depleting agent since it does not appear to possess secondary toxicities [2].

GSH may also play a role in the synthesis of vasoactive arachidonic acid metabolites. Inhibition of cyclooxygenase activity by GSH in the presence of excess GSH peroxidase was first demonstrated by Smith and Lands [10]. Depletion of GSH with BSO has been shown to be associated with increased synthesis of 6-ketoPGF$_{1\alpha}$ (the stable metabolite of prostacyclin) in macrophages [11], endothelial cells [12], and rabbit aortic rings [8]. Thus, acute depletion of GSH may alter tissue blood flow via increased prostacyclin synthesis [6, 13]. Recently, Bottje *et al.* [6] presented evidence that DEM-mediated GSH depletion was associated with increased plasma 6-ketoPGF$_{1\alpha}$ and TxB_2 levels in anesthetized rabbits and hypothesized that a critical level of GSH depletion may be required to stimulate synthesis of these prostanoids. However, in a preliminary study, Maynard *et al.* [14] reported no change in tissue or plasma 6-ketoPGF$_{1\alpha}$ levels following BSO-mediated depletion of hepatic GSH.

Few studies have investigated the relationships between BSO- or DEM-mediated alterations in GSH and tissue or plasma prostanoid production *in vivo*. Therefore, the major objective of this study was to establish relationships between GSH, prostacyclin, and thromboxane in tissues and plasma from rats treated with DEM or BSO. These studies were conducted with and without pretreatment of animals with 3-methylcholanthrene (3-MC) which has been reported to increase lipid peroxidation [15] which in turn may affect cyclooxygenase activity [16–18].

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats were obtained from the Charles River Breeding Laboratories (Wilmington, MA). The rats were maintained three to a cage on a 12-hr light, 12-hr dark photoperiod, and provided food and water *ad lib*. The rats weighed 530 ± 80 g at the time of the experiments.

Chemicals

BSO was obtained from the Chemical Dynamics Corp. (South Plainfield, NJ). All other chemicals

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‡ Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; BSO, buthionine sulfoximine; DEM, diethyl maleate; 6-ketoPGF$_{1\alpha}$, prostacyclin; TxB_2, thromboxane; 3-MC, 3-methylcholanthrene; and RIA, radioimmunoassay.

were obtained from the Sigma Chemical Co. (St. Louis, MO).

Experimental protocol

Experiments were carefully timed so that all samples were obtained between 2:00 and 4:00 p.m. each day to reduce differences that might occur due to diurnal fluctuations in GSH [19].

Expt. 1. The objective of Expt. 1 was to determine the effects of different doses of BSO on tissue GSH and tissue and plasma 6-ketoPGF$_{1\alpha}$ and TxB$_2$. Three groups of rats were utilized in which rats were randomly assigned to either 0.4, 0.8 or 1.2 g BSO/kg body wt or as a paired saline control. Each concentration of BSO was diluted in saline to a final volume of 8.0 mL/kg body wt and buffered to pH 6.7 to 7.0. The paired control received saline at 8.0 mL/kg body wt. Rats were injected (i.p.) and maintained for 4 hr to allow maximal GSH depletion [7], after which surgical procedures were performed.

Pairs of rats (a BSO-treated and respective control) were anesthetized with an i.m. injection of ketamine hydrochloride (40 mg/kg body wt) and xylazine (4 mg/kg body wt). A cannula was inserted into the jugular vein for infusion of physiological saline (0.05 mL/min/kg body wt) and sodium pentobarbital as required to maintain anesthesia. Cannulae were also inserted into the carotid artery and portal vein (via abdominal incision). The incision was closed and the rats were allowed 30 min to stabilize. The total time period from induction of anesthesia to blood and tissue sampling was 60–70 min.

Portal (2.5 mL) and arterial (4.0 mL) blood samples were collected directly into ice-cold plastic tubes containing an aspirin–heparin–saline solution (100 U heparin/mL saline) to give a final concentration of 0.48 mmol aspirin. The blood was centrifuged at 5° and 5000 rpm for 2.5 min in a microfuge (Hema-C, Jouan, Paris, France). Plasma was frozen immediately in liquid nitrogen and stored at −80° for radioimmunoassay (RIA) determinations of 6-ketoPGF$_{1\alpha}$ and TxB$_2$.

After the blood samples were collected, the animals were euthanized with sodium pentobarbital. A portion of the liver and lung, and the entire kidney, spleen, and heart were quickly removed and frozen in liquid nitrogen for subsequent determination of GSH, 6-ketoPGF$_{1\alpha}$ and TxB$_2$ content.

Expt. 2. The results of Expt. 1 indicated that depletion of GSH by BSO to approximately 34% of control had no effect on plasma or tissue prostanoids. Therefore, Expt. 2 was designed to determine the effects of DEM or BSO, with and without pretreatment with 3-MC to induce cytochrome P450, on GSH and prostanoids. Also, to avoid potential surgical complications which may have occurred in Expt. 1, tissue and blood sampling in Expt. 2 were made immediately after animals were anesthetized.

Each GSH-depleting agent or its control was given in combination with 3-MC or the corn oil carrier in a 2 × 2 factorial experiment. Rats were randomly assigned to treatments in each experiment resulting in seven rats per treatment. Rats received either 3-MC dissolved in corn oil and injected i.p. at 35 mg/kg in 1.0 mL corn oil/kg body wt, or an equal volume of the corn oil carrier. Injections were daily for 3 consecutive days. Twenty-four hours after the last 3-MC or corn oil injection, rats were treated with DEM, BSO, or the appropriate carrier as described below.

In Expt. 2A, rats were injected (i.p.) with DEM (1.0 mL/kg body wt diluted 1:1 with corn oil) or corn oil vehicle alone (2 mL/kg body wt). After the oil or DEM injection, the rats were returned to their cages with food and water for a 1-hr period prior to sampling. In Expt. 2B, rats were injected (i.p.) with BSO (0.8 g L-BSO/kg body wt in 8 mL saline/kg body wt, pH 6.7 to 7.0) or saline vehicle alone (8 mL/kg body wt). After the saline or BSO injection, the rats were returned to their cages with food and water for a 4-hr period prior to sampling.

Following the respective GSH depletion periods for DEM and BSO, rats were anesthetized with ketamine hydrochloride (40 mg/kg body wt) and xylazine (0.4 mg/kg body wt). When the animal reached a surgical plane of anesthesia (4–5 min later), an incision was made to expose the heart and internal organs. A blood sample (2.5 mL) was withdrawn from the right ventricle into a syringe containing an ice-cold aspirin–heparin–saline solution (0.5 mL, 100 U heparin/mL) to give a final concentration of 0.48 mM aspirin. Immediately after blood sampling the medial lobe of the liver, and the kidneys, spleen, heart, and lungs were removed and frozen in liquid nitrogen.

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

Tissues were homogenized in an aspirin solution to a final concentration of 0.48 mmol [20]. The homogenate was centrifuged at 2500 rpm for 20 min, and RIA analysis was performed on the supernatant.

Tissue and plasma levels of 6-ketoPGF$_{1\alpha}$ and/or TxB$_2$ (the stable metabolites of prostacyclin and thromboxane, respectively) were determined using a sequential double antibody procedure equilibrated at 4° [21–23]. The procedure used tritiated metabolites (Dupont Chemical Co., Boston, MA). The primary antibodies have been examined extensively; cross-reactivity with other prostaglandins was less than 1% with the exception of PGF$_{2\alpha}$ which cross-reacts with the 6-ketoPGF$_{1\alpha}$ antibody at 1.7% and PGD$_2$ which cross-reacts with the TxB$_2$ antibody at 3.9% [23]. Additionally, the 11-dehydroTXB$_2$ and the 6,15-diketo-13,14-dihydroPGF$_{1\alpha}$ metabolites cross-react less than 0.1%.* All values were determined with a standard curve and calculated using specific RIA software (SECURIA, Packard Instrument Co., Downers Grove, IL).

Glutathione analysis

Tissue concentrations of GSH and oxidized glutathione (GSSG) were determined by HPLC according to Fariss and Reed [24]. This method employs the perchloric acid precipitation of proteins followed by reaction of iodoacetic acid with thiols to form *S*-carboxymethyl derivatives and

* Mathias MM, personal communication, cited with permission.

Table 1. Effect of 0.4 g/kg buthionine sulfoximine (BSO) on hepatic, renal, pulmonary, splenic, and coronary concentrations of reduced glutathione (GSH), oxidized glutathione (GSSG), 6-ketoPGF$_{1\alpha}$, and TxB$_2$ in male rats

	GSH (μmol/g)	GSSG (μmol/g)	6-ketoPGF$_{1\alpha}$ (ng/g)	TxB$_2$ (ng/g)
Liver				
Control	4.88 ± 0.27	0.25 ± 0.02	4.36 ± 0.61	2.45 ± 0.74
BSO 0.4	2.19 ± 0.27*	0.19 ± 0.02	4.88 ± 0.61	3.09 ± 0.74
% of Control	45	76	112	126
Kidney				
Control	0.28 ± 0.02	ND†	36.17 ± 17.22	2.61 ± 0.48
BSO 0.4	0.17 ± 0.02*	ND	10.61 ± 17.22	1.35 ± 0.48
% of Control	61	ND	29	52
Pulmonary				
Control	1.28 ± 0.05	0.24 ± 0.01	47.55 ± 13.18	4.90 ± 1.59
BSO 0.4	1.03 ± 0.05*	0.16 ± 0.01*	53.31 ± 13.18	7.11 ± 1.59
% of Control	80	67	112	145
Spleen				
Control	0.42 ± 0.08	0.65 ± 0.11	22.40 ± 3.54	43.33 ± 5.85
BSO 0.4	0.31 ± 0.08	0.39 ± 0.11	20.36 ± 3.54	25.69 ± 5.85
% of Control	74	60	91	59
Heart				
Control	1.48 ± 0.12	0.22 ± 0.02	6.12 ± 1.52	0.70 ± 0.09
BSO 0.4	1.49 ± 0.12	0.23 ± 0.02	3.28 ± 1.52	0.63 ± 0.09
% of Control	101	105	54	90

Each value is the mean ± SEM of 5 observations expressed in units per gram tissue weight. Values were determined in tissues obtained 4 hr after i.p. injection of 0.4 g L-BSO/kg body wt in saline (0.1 g/mL) or saline vehicle.

* Significantly different from control ($P < 0.05$).

† None detected.

derivatization of amino groups in the supernatant with 1-fluoro-2,4-dinitrobenzene. Derivatized thiols were then separated with an ion-exchange column (3-aminopropyl-spherisorb column, 25 cm × 4.6 mm, 5 μm, Custom Columns, Inc., Houston, TX) using an ISCO model 2350 pump, model 2360 gradient programmer, and a v^4 variable wavelength detector (ISCO, Lincoln, NE).

Statistics

Data are presented as means ± SEM. Differences in mean values for each parameter within a tissue were assessed by analysis of variance using the general linear model procedure of SAS [25] and considered different at $P < 0.05$. Mean values within a tissue parameter with unequal variances were assessed by multiple *t*-tests and considered different at $P < 0.05$.

RESULTS

Since hepatic GSH levels in control rats in Expt. 1 were different between each group of rats (Tables 1–3), the data are presented separately. Control values for other tissues did not differ between experiments. The reason for the different hepatic GSH values for the paired controls was not apparent. All rats were handled similarly (i.e. anesthesia, surgery, etc.), and there were no differences in time required for surgical preparation and stabilization.

BSO lowered ($P < 0.05$) hepatic GSH to 45, 34 and 38% of control for 0.4, 0.8, and 1.2 g L-BSO/kg body wt, respectively (Tables 1–3). With 0.4 g/kg body wt), BSO lowered ($P < 0.05$) renal and pulmonary GSH and pulmonary GSSG in comparison to controls (Table 1). Besides the effect on liver GSH and GSSG, there were no differences in GSH or GSSG values in kidney, spleen or heart tissue between controls and rats treated with 0.8 g BSO/kg body wt (Table 2). Pulmonary tissue was not obtained in this group of rats. Besides depleting hepatic GSH, hepatic GSSG levels were lower in rats receiving 1.2 g BSO/kg body wt in comparison to controls (Table 3). Similar to the 0.4 g/kg dose, pulmonary GSH was lowered ($P < 0.05$) by BSO in comparison to controls following 1.2 g BSO/kg. Spleen GSH was inexplicably higher ($P < 0.05$) in BSO-treated rats compared to controls (Table 3).

RIA analysis of tissue 6-ketoPGF$_{1\alpha}$ and tissue TxB$_2$ revealed no differences at any level of BSO in comparison to the control, with the exception of 1.2 g BSO/kg dose which lowered ($P < 0.05$) TxB$_2$ levels in renal tissue (Tables 1–3). Portal and arterial plasma 6-ketoPGF$_{1\alpha}$ levels were also unaffected by

Table 2. Effect of 0.8 g/kg buthionine sulfoximine (BSO) on hepatic, renal, splenic, and coronary concentrations of reduced glutathione (GSH), oxidized glutathione (GSSG), 6-ketoPGF$_{1\alpha}$, and TxB_2 in male rats

	GSH (μmol/g)	GSSG (μmol/g)	6-ketoPGF$_{1\alpha}$ (ng/g)	TxB_2 (ng/g)
Liver				
Control	3.17 ± 0.17	0.24 ± 0.01	9.36 ± 1.24	2.84 ± 0.59
BSO 0.8	1.08 ± 0.17*	0.19 ± 0.01*	10.81 ± 1.24	2.70 ± 0.59
% of Control	34	79	115	95
Kidney				
Control	0.23 ± 0.04	ND†	12.85 ± 2.01	1.74 ± 0.17
BSO 0.8	0.22 ± 0.04	ND	13.72 ± 2.01	1.71 ± 0.17
% of Control	96	ND	107	98
Spleen				
Control	0.20 ± 0.04	0.41 ± 0.05	31.16 ± 7.78	35.25 ± 4.10
BSO 0.8	0.22 ± 0.04	0.30 ± 0.05	30.86 ± 7.78	32.29 ± 4.10
% of Control	110	73	99	92
Heart				
Control	1.54 ± 0.13	0.27 ± 0.02	5.82 ± 2.77	1.23 ± 0.19
BSO 0.8	1.46 ± 0.13	0.25 ± 0.02	11.66 ± 2.77	1.41 ± 0.19
% of Control	95	93	200	115

Each value is the mean ± SEM of 5 observations expressed in units per gram tissue weight. Values were determined in tissues obtained 4 hr after i.p. injection of 0.8 g L-BSO/kg body wt in saline (0.1 g/mL) or saline vehicle.

* Significantly different from control ($P < 0.05$).

† None detected.

Table 3. Effect of 1.2 g/kg buthionine sulfoximine (BSO) on hepatic, renal, pulmonary, splenic, and coronary concentrations of reduced glutathione (GSH), oxidized glutathione (GSSG), 6-ketoPGF$_{1\alpha}$ and TxB_2 in male rats

	GSH (μmol/g)	GSSG (μmol/g)	6-ketoPGF$_{1\alpha}$ (ng/g)	TxB_2 (ng/g)
Liver				
Control	3.94 ± 0.14	0.21 ± 0.01	3.86 ± 0.90	1.63 ± 0.37
BSO 1.2	1.48 ± 0.14*	0.10 ± 0.01*	6.02 ± 0.90	1.66 ± 0.37
% of Control	38	48	156	102
Kidney				
Control	0.27 ± 0.04	ND†	15.71 ± 2.99	1.89 ± 0.19
BSO 1.2	0.19 ± 0.04	ND	12.41 ± 2.99	1.22 ± 0.19*
% of Control	70	ND	79	65
Pulmonary				
Control	1.31 ± 0.15	0.17 ± 0.02	80.10 ± 19.78	6.13 ± 0.82
BSO 1.2	0.77 ± 0.15*	0.16 ± 0.02	71.05 ± 19.78	5.67 ± 0.82
% of Control	59	94	89	93
Spleen				
Control	0.22 ± 0.16	0.46 ± 0.07	28.56 ± 6.50	33.48 ± 5.72
BSO 1.2	0.81 ± 0.16*	0.35 ± 0.07	28.89 ± 6.50	31.09 ± 5.72
% of Control	368	76	101	93
Heart				
Control	1.56 ± 0.10	0.26 ± 0.05	5.38 ± 0.97	0.66 ± 0.20
BSO 1.2	1.31 ± 0.10	0.25 ± 0.05	4.84 ± 0.97	0.84 ± 0.20
% of Control	84	96	90	127

Each value is the mean ± SEM of 5 observations expressed in units per gram tissue weight. Values were determined in tissues obtained 4 hr after i.p. injection of 1.2 g L-BSO/kg body wt in saline (0.1 g/mL) or saline vehicle.

* Significantly different from control ($P < 0.05$).

† None detected.

Table 4. Effects of diethyl maleate (DEM) and buthionine sulfoximine (BSO) with or without pretreatment of 3-methylcholanthrene (3-MC) on hepatic tissue concentrations of reduced glutathione (GSH), 6-ketoPGF$_{1\alpha}$ (6-Keto), and TxB$_2$ in male rats

		Expt. 2A		Expt. 2B	
	Inducer*	Oil	DEM	Saline	BSO
GSH†	3-MC	5.77 ± 0.32^{c}	0.56 ± 0.32^{a}	6.37 ± 0.32^{c}	3.23 ± 0.32^{b}
(μmol/g)	Oil	5.48 ± 0.32^{c}	0.88 ± 0.32^{a}	6.32 ± 0.32^{c}	2.97 ± 0.32^{b}
6-Keto	3-MC	6.95 ± 1.74^{c}	46.06 ± 12.30^{d}	3.59 ± 0.50^{bc}	5.27 ± 0.44^{c}
(ng/g)	Oil	2.48 ± 0.69^{ab}	26.66 ± 4.92^{d}	2.27 ± 0.21^{a}	3.22 ± 0.33^{b}
TxB$_2$	3-MC	1.79 ± 0.24^{c}	1.54 ± 0.24^{bc}	1.52 ± 0.24^{bc}	1.70 ± 0.24^{c}
(ng/g)	Oil	1.64 ± 0.24^{c}	1.21 ± 0.24^{abc}	0.68 ± 0.24^{a}	0.89 ± 0.24^{ab}

Each value is the mean ± SEM of 6–7 observations expressed in units per gram wet tissue weight. Values were determined in tissue obtained 1 hr after i.p. injection of DEM (1 mL/kg body wt, diluted 1:1 with corn oil) or corn oil vehicle (2 mL/kg body wt) or 4 hr after i.p. injection of L-BSO (0.8 g/kg body wt in saline 0.1 g/mL) or saline vehicle.

* 3-Methylcholanthrene was injected (i.p.) daily at 35 mg/kg body wt in corn oil (35 mg/mL) for 3 consecutive days prior to administration of DEM or BSO.

† GSSG levels were less than 2% of GSH with many samples being below limits of sensitivity.

[abcd] Mean values within a tissue parameter with different superscripts are significantly different ($P < 0.05$).

BSO (data not shown). However, when data from controls and BSO treatments were pooled, portal plasma 6-ketoPGF$_{1\alpha}$ levels were 0.75 ± 0.08 ng/mL and higher ($P < 0.05$) than the 0.33 ± 0.04 ng/mL in arterial plasma. Plasma TxB$_2$ levels were not determined in either Expt. 1 or 2 since platelet activation was likely to occur with the procedures employed in this study.*

The effects of DEM and BSO in conjunction with 3-MC in Expt. 2 on hepatic, renal, and pulmonary GSH, 6-ketoPGF$_{1\alpha}$, and TxB$_2$ are presented in Tables 4, 5 and 6, respectively. In general, while both DEM and BSO depleted GSH ($P < 0.05$) in all tissues, significant elevations in 6-ketoPGF$_{1\alpha}$ and TxB$_2$ were observed only following DEM treatment. 3-MC in conjunction with DEM and BSO had no effect on malondialdehyde (thiobarbituric reactive substance, as an index of lipid peroxidation, data not shown).

In Expt. 2A, DEM had lowered ($P < 0.05$) hepatic GSH 1 hr after treatment to 10 and 16% of controls in rats pretreated with 3-MC or corn oil, respectively (Table 4). This contrasts with hepatic GSH depletion of 51 and 47% obtained 4 hr after BSO injection in rats pretreated with 3-MC or corn oil in Expt. 2B. In both Expt. 2A and 2B, hepatic GSSG levels were less than 2% of GSH (data not shown).

Maximal depletion of GSH by BSO has been reported to occur between 2 and 4 hr following administration with little changes in hepatic GSH being observed between 2 and 6 hr [2, 7]. For this reason, the 4-hr treatment period was chosen in the present study. The depletion of hepatic GSH to 34% of controls in Expt. 1 was similar to previous reports [2, 9] while the 50% depletion in Expt. 2 was nearly identical to that achieved in rabbits 7 hr after BSO treatment [8]. The extent of depletion by DEM in the present study concurs with previous reports [3–5].

The greater percent depletion of hepatic GSH by the 0.8 g dose of BSO in Expt. 1 in comparison to Expt. 2B could be attributed to the longer depletion period since tissues were obtained after an additional 60- to 70-min period for surgical preparation following the 4-hr depletion period. However, the total amount of change in GSH was greater in Expt. 2B (~3.2 μmol/g, Table 4) in comparison to 2.1 μmol/g change in Expt. 1 (Table 2). The greater change in GSH in Expt. 2B could be related to the higher control hepatic GSH values.

In Expt. 2A, DEM caused an increase ($P < 0.05$) in hepatic 6-ketoPGF$_{1\alpha}$ levels in rats with or without prior 3-MC treatment (Table 4). BSO had no effect on hepatic 6-ketoPGF$_{1\alpha}$ tissue levels in animals pretreated with 3-MC but a slight increase ($P < 0.05$) was noted following BSO in animals that had received the corn oil vehicle. DEM with or without 3-MC had no effect on hepatic TxB$_2$ levels but BSO elevated ($P < 0.05$) tissue TxB$_2$ levels in rats pretreated with 3-MC.

Renal parameters for Expt. 2A and 2B are presented in Table 5. In Expt. 2A, DEM in animals pretreated with 3-MC and corn oil depleted renal GSH to 56 and 48%, respectively, after 1 hr in comparison to controls. In contrast, BSO lowered ($P < 0.05$) GSH levels to 25% of controls after 4 hr regardless of pretreatment. As in hepatic tissue, 6-ketoPGF$_{1\alpha}$ levels were increased ($P < 0.05$) by DEM treatment but not by BSO treatment. Renal TxB$_2$ levels were also increased ($P < 0.05$) by DEM.

Pulmonary GSH levels were depleted by DEM to 35 and 27% of controls in rats pretreated with 3-MC and corn oil, respectively (Table 6). In contrast, BSO with or without 3-MC depleted ($P < 0.05$) pulmonary GSH slightly to approximately 85% of controls. GSSG levels were lowered by DEM but

* Mathias MM, personal communication, cited with permission.

Table 5. Effects of diethyl maleate (DEM) and buthionine sulfoximine (BSO) with or without pretreatment of 3-methylcholanthrene (3-MC) on renal tissue concentrations of reduced glutathione (GSH), 6-ketoPGF$_{1\alpha}$ (6-Keto), and TxB$_2$ in male rats

		Expt. 2A		Expt. 2B	
	Inducer*	Oil	DEM	Saline	BSO
GSH†	3-MC	2.04 ± 0.08^{d}	1.15 ± 0.08^{b}	2.62 ± 0.08^{c}	0.66 ± 0.08^{a}
(μmol/g)	Oil	1.71 ± 0.08^{c}	0.83 ± 0.08^{a}	2.47 ± 0.08^{c}	0.61 ± 0.08^{a}
6-Keto	3-MC	22.06 ± 4.27^{b}	51.03 ± 4.27^{c}	9.69 ± 4.61^{a}	13.50 ± 4.61^{ab}
(ng/g)	Oil	16.18 ± 4.27^{ab}	51.59 ± 4.27^{c}	9.11 ± 4.27^{a}	11.36 ± 4.27^{ab}
TxB$_2$	3-MC	1.80 ± 0.22^{b}	3.19 ± 0.22^{c}	0.59 ± 0.22^{a}	0.57 ± 0.23^{a}
(ng/g)	Oil	1.39 ± 0.22^{b}	2.65 ± 0.22^{c}	0.39 ± 0.22^{a}	0.61 ± 0.22^{a}

Each value is the mean ± SEM of 6–7 observations expressed in units per gram wet tissue weight. Values were determined in tissue obtained 1 hr after i.p. injection of DEM (1 mL/kg body wt, diluted 1:1 with corn oil) or corn oil vehicle (2 mL/kg body wt) or 4 hr after i.p. injection of L-BSO (0.8 g/kg body wt in saline 0.1 g/mL) or saline vehicle.

* 3-Methylcholanthrene was injected (i.p.) daily at 35 mg/kg body wt in corn oil (35 mg/mL) for 3 consecutive days prior to administration of DEM or BSO.

† GSSG levels were less than 2% of GSH with many samples being below limits of sensitivity.

^{abcde} Mean values within a tissue parameter with different superscripts are significantly different ($P < 0.05$).

Table 6. Effects of diethyl maleate (DEM) and buthionine sulfoximine (BSO) with or without pretreatment of 3-methylcholanthrene (3-MC) on pulmonary tissue concentrations of reduced glutathione (GSH), 6-ketoPGF$_{1\alpha}$ (6-Keto), and TxB$_2$ in male rats

		Expt. 2A		Expt. 2B	
	Inducer*	Oil	DEM	Saline	BSO
GSH	3-MC	1.55 ± 0.06^{c}	0.54 ± 0.06^{a}	1.79 ± 0.06^{d}	1.54 ± 0.06^{c}
(μmol/g)	Oil	1.42 ± 0.06^{bc}	0.39 ± 0.06^{a}	1.51 ± 0.06^{c}	1.28 ± 0.06^{b}
GSSG	3-MC	0.21 ± 0.02^{bc}	0.07 ± 0.02^{a}	0.24 ± 0.02^{c}	0.21 ± 0.02^{bc}
(μmol/g)	Oil	0.20 ± 0.02^{bc}	0.04 ± 0.02^{a}	0.21 ± 0.02^{bc}	0.16 ± 0.02^{b}
6-Keto	3-MC	173.49 ± 46.9^{ab}	59.72 ± 136.0^{c}	78.16 ± 12.8^{a}	86.73 ± 29.5^{a}
(ng/g)	Oil	119.89 ± 47.3^{ab}	278.11 ± 73.4^{bc}	88.69 ± 23.0^{a}	71.43 ± 10.4^{a}
TxB$_2$	3-MC	12.70 ± 2.17^{bc}	16.77 ± 2.17^{c}	7.85 ± 2.17^{ab}	6.96 ± 2.17^{ab}
(ng/g)	Oil	9.31 ± 2.17^{ab}	15.59 ± 2.17^{c}	5.58 ± 2.17^{a}	4.53 ± 2.17^{a}

Each value is the mean ± SEM of 6–7 observations expressed in units per gram wet tissue weight. Values were determined in tissue obtained 1 hr after i.p. injection of DEM (1 mL/kg body wt, diluted 1:1 with corn oil) or corn oil vehicle (2 mL/kg body wt) or 4 hr after i.p. injection of L-BSO (0.8 g/kg body wt in saline 0.1 g/mL) or saline vehicle.

* 3-Methylcholanthrene was injected (i.p.) daily at 35 mg/kg body wt in corn oil (35 mg/mL) for 3 consecutive days prior to administration of DEM or BSO.

^{abcd} Mean values within a tissue parameter with different superscripts are significantly different ($P < 0.05$).

not by BSO, regardless of pretreatment. Pulmonary 6-ketoPGF$_{1\alpha}$ levels were increased by DEM, although the increase was only significant with 3-MC pretreatment. BSO had no effect on pulmonary 6-ketoPGF$_{1\alpha}$ or TxB$_2$ levels. TxB$_2$ levels in pulmonary tissue were increased ($P < 0.05$) by DEM in rats pretreated with corn oil.

The effects of the various treatments on plasma levels of 6-ketoPGF$_{1\alpha}$ are shown in Fig. 1. Plasma 6-ketoPGF$_{1\alpha}$ levels were elevated ($P < 0.05$) by DEM but plasma levels were significantly greater in rats that had not received 3-MC pretreatment. GSH depletion by BSO had no effect on plasma 6-ketoPGF$_{1\alpha}$ levels.

DISCUSSION

Mammalian hepatic GSH values generally range from 6–8 μmol/g with renal values being approximately 2–3 μmol/g [2, 7, 26]. The cystathionine pathway is responsible in part for the maintenance of relatively high GSH values in the liver [27, 28]. The kidney is one of the major utilizers of plasma GSH [29]. Although there were no differences in

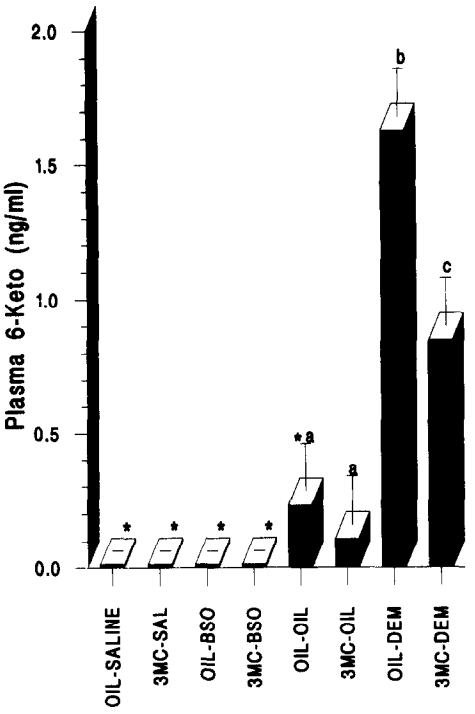

Fig. 1. Effect of buthionine sulfoximine (BSO) or diethyl maleate (DEM) with and without 3-methylcholanthrene (3-MC) pretreatment on plasma 6-ketoPGF$_{1\alpha}$ levels in male rats. Each value is the mean ± SEM of 6 or 7 observations. Key: (*) four or more values were below the level of detection; and (abc) mean values with different letters are significantly different ($P < 0.05$).

kidney, spleen, heart, and/or lung GSH values for the paired controls in Expt. 1, the fact that hepatic GSH levels differed between each experiment, and that hepatic and renal GSH values were considerably lower than those reported in the literature, indicates that considerable differences in GSH metabolism existed in Expt. 1.

The low GSH values in the liver and kidney in Expt. 1 might be due to the surgical stress or duration of anethesia. The liver maintains a high rate of GSH efflux even when hepatic GSH levels are below normal [29, 30]. Lower hepatic GSH levels could affect tissues with high GSH turnover rates, such as the kidney, most severely, due to their dependence on plasma GSH [31, 32]. Thus, it is reasonable to propose that the abdominal surgery and stabilization period in Expt. 1 resulted in depletion of hepatic, and especially, renal GSH. This hypothesis appears to be supported since hepatic and renal GSH levels in rats in Expt. 2 that were anesthetized for less than 5 min were comparable to previously reported values. Surgical stress also may have accounted for low hepatic GSH values in pigs [33] while surgical procedures and anesthesia did not appear to affect hepatic GSH levels in rabbits [6]. Thus, the effects of surgery and/or anesthesia may depend on the species being studied.

The elevation in plasma 6-ketoPGF$_{1\alpha}$ in DEM-treated rats (Fig. 1) in Expt. 2 concurs with a recent report in anesthetized rabbits [6]; plasma levels of 6-ketoPGF$_{1\alpha}$ observed in the present study were 10- to 20-fold lower, however. Rats pretreated with 3-MC exhibited significantly lower plasma levels of 6-ketoPGF$_{1\alpha}$ in comparison to non-induced rats (Fig. 1). The reason for lower circulating 6-ketoPGF$_{1\alpha}$ levels is therefore not apparent since hepatic and pulmonary tissue 6-ketoPGF$_{1\alpha}$ levels were numerically twice as high in 3-MC/DEM-treated rats compared to those receiving corn oil carrier vehicle prior to DEM treatment.

Tissue levels of prostanoids in the present study reflect both intrinsic endogenous concentrations as well as that contributed by blood remaining in the tissue at freezing. Thus, as shown, basal levels of renal 6-ketoPGF$_{1\alpha}$ were greater than hepatic, presumably reflecting renal medullary prostaglandin synthesis [34] but were less than pulmonary which is known for high levels of 6-ketoPGF$_{1\alpha}$ synthesis from pulmonary endothelium [35]. Furthermore, the relatively greater increase in hepatic tissue 6-ketoPGF$_{1\alpha}$ levels following DEM treatment (Table 4) in comparison to renal tissue (Table 5) could be due to the portal component of hepatic blood flow since splanchnic vasculature synthesizes large amounts of prostacyclin [36–38].

Bottje *et al.* [6] hypothesized that a critical level of hepatic GSH depletion may be required in order to stimulate prostacyclin synthesis. Possibly, depleting GSH to a critical level is necessary to elevate peroxide tone and stimulate cyclooxygenase activity [16, 17]. Thus, the greater depletion of GSH by DEM may have been sufficient to stimulate cyclooxygenase activity, whereas the depletion to 34% of control by BSO was insufficient. This hypothesis appears to be supported in the present study in plasma, hepatic and pulmonary tissue but not in renal tissue in which BSO depleted renal GSH to a greater extent than DEM without altering 6-ketoPGF$_{1\alpha}$ levels. The results indicate a possible tissue specificity, or that factors other than GSH depletion alone are required to increase 6-ketoPGF$_{1\alpha}$ levels.

The lack of effect of BSO on prostacyclin in the present study differs from previous reports in which prostacyclin synthesis was elevated following BSO [8, 11, 12]. Depletion of GSH content in macrophages to 3% of control after 16 hr of BSO treatment produced a 90–95% increase in 6-ketoPGF$_{1\alpha}$ synthesis with an equal and concomitant reduction in PGE_2 synthesis; total synthesis of arachidonic acid metabolites was unchanged [11]. LaSierra *et al.* [8] indicated an inverse relationship between hepatic GSH and 6-ketoPGF$_{1\alpha}$ synthesis in rabbit aortic rings with significant increases in 6-ketoPGF$_{1\alpha}$ occurring when hepatic GSH had been depleted to only 50% of control. Finally, Buckley *et al.* [12] reported a clear dose–response relationship between GSH depletion with BSO and 6-ketoPGF$_{1\alpha}$ synthesis in first passaged porcine aorta endothelial cells.

Some reports indicate that DEM does not adversely affect hepatic function [39, 40]. However, DEM has been shown to produce alterations in microsomal monooxygenase activity [41, 42], metabolic acidosis [6, 43], impaired glycogen metab-

olism [44], decreased protein synthesis [45] and Na^+/K^+-ATPase activity [46, 47]. Therefore, the effect of DEM on prostacyclin synthesis may be due to a factor(s) other than GSH depletion alone.

In summary, GSH depletion by DEM caused an increase in 6-ketoPGF$_{1\alpha}$ in the plasma, liver, kidney, and lungs. TxB_2 levels were increased by DEM in the kidney and in pulmonary tissue of rats that had not been pretreated with 3-MC. BSO depletion of GSH after 4 hr had no effect on tissue or plasma 6-ketoPGF$_{1\alpha}$ levels with the exception of a small but significant increase in hepatic 6-ketoPGF$_{1\alpha}$ levels in non-induced rats. Thus, there may be an additional factor(s) introduced by DEM treatment which stimulates prostaglandin synthesis that is not associated with BSO. Studies are currently being conducted to attempt to identify this factor.

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