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INDOMETHACIN ATTENUATION OF CELIAC BLOOD FLOW HYPEREMIA FOLLOWING GLUTATHIONE DEPLETION*

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Abstract—The effect of glutathione (GSH) depletion on mean celiac blood flow (MCBF) was determined in domestic fowl. Diethyl maleate (DEM, 1 mL/kg body wt) decreased hepatic and duodenal GSH to approximately 15% of control. This GSH depletion was associated with an increase in MCBF and decreases in mean arterial blood pressure (MABP) and celiac vascular resistance (CVR). While indomethacin attenuated the rise in MCBF, this cyclooxygenase inhibitor had no effect on the decrease of MABP or CVR which occurred following DEM treatment. The results indicate that GSH depletion may increase vasodilatory prostaglandin synthesis since elevations in MCBF were attenuated by cyclooxygenase inhibition.

Glutathione (GSH‡) is a ubiquitous intracellular compound with a broad range of vital functions which include detoxification and protection of cells from reactive oxygen species [1, 2]. Depletion of tissue GSH by various agents has proven to be a useful tool in xenobiotic studies. Treatment of animals with diethyl maleate (DEM) results in a profound decrease (within 45–60 min) in liver GSH concentrations and, to a lesser degree, in other tissues in several mammalian species [1, 3–6].

Glutathione is also known to be important in regulating arachidonic acid metabolism [7–9]. Depletion of GSH has been associated with increased prostacyclin synthesis in macrophages [9] and rabbit aortic rings [10]. Since prostacyclin is known to increase intestinal [11] and portal venous [12] blood flow, it is possible that elevations in total hepatic perfusion following GSH depletion noted by Bottje *et al.* [13] resulted from increased synthesis of vasodilatory prostaglandins. An increase in portal venous flow following GSH depletion could result from intestinal vasodilation and be detected as an increase in intestinal arterial flow. Furthermore, if this vasodilation resulted from increased prostaglandin synthesis, cyclooxygenase inhibition should block or reduce any hyperemic response to GSH depletion. Few studies have focused on relationships between GSH and hemodynamics *in vivo* and none have been carried out in domestic fowl. Thus, the objectives of this study were to determine the effect of GSH depletion on blood flow in the celiac artery in the domestic fowl and to determine if this vascular response could be attenuated by pretreatment of animals with indomethacin, to inhibit cyclooxygenase.

MATERIALS AND METHODS

Animals. Male broilers (3–4 kg body wt) were maintained in cages (30 × 45 × 40 cm) for at least 2 weeks prior to experimentation. Birds were provided feed and water *ad lib.* and maintained in thermoneutral conditions (22–25°, 40–60% relative humidity).

Surgical preparation. For blood flow studies, birds were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) with supplemental doses given at 30- to 45-min intervals as required. An electromagnetic blood flow probe (Zepeda Instruments, Inc., Seattle, WA) and vascular occluder (*In Vivo* Metrics, Healdsburg, CA) were placed on the celiac artery and a cannula was inserted in the left carotid artery as described previously [14]. Blood flow probes were calibrated *in vitro* using modified methods described by Roman-Ponce *et al.* [15].

Experimental protocol. After surgical preparation, the birds were allowed a 20- to 30-min stabilization period. Prior to each experiment, an occlusive zero was obtained and the flow meter was adjusted to electronic zero. A second occlusive zero at the end of each experiment revealed that electronic drift was negligible.

The objective of Expt. 1 was to determine the effect of GSH depletion on mean celiac blood flow (MCBF). After a 30-min initial control period, birds (N = 10) were injected with DEM (1 mL/kg body wt, i.p.), and physiological variables were monitored for an additional 60-min period. In each experiment, MCBF (mL/min/kg body wt) and mean arterial blood pressure (MABP, mmHg) were recorded at 10-min intervals on a strip chart recorder. The MABP was obtained by electronically damping the pulsatile signal from the pressure transducer (P31ID, Gould-Statham, Hato Rey, Puerto Rico). Celiac vascular resistance (CVR) was calculated as MABP/

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‡ Abbreviations: GSH, glutathione; MCBF, mean celiac blood flow; DEM, diethyl maleate; MABP, mean arterial blood pressure; and CVR, celiac vascular resistance.

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MCBF. At the end of each experiment, birds were killed by an overdose of sodium pentobarbital.

To determine the effect of DEM on hepatic and duodenal glutathione (GSH) content, two additional groups of birds were anesthetized and treated with DEM or sesame oil (1 mL/kg body wt). Fifty minutes later, birds were killed and portions of the liver and proximal 20–30 cm of duodenum were quickly removed and frozen in liquid nitrogen. Prior to freezing, the duodenum was cut lengthwise and rinsed in cold saline to remove lumen contents. Tissue GSH content was determined as described below.

Jimenez *et al.* [6] noted that a profound metabolic acidosis develops in animals treated with DEM. To avoid potential disruption of hemodynamics during blood flow studies which could occur with blood sampling [16, 17], Expt. 2 was performed on a separate group of birds to determine the effect of DEM on blood lactate and acid–base balance. Birds were anesthetized and implanted with a cannula in the carotid artery as described above. After a 30-min control period, birds were injected with DEM (N = 4) or sesame oil (N = 4) (1 mL/kg body wt, i.p.). Blood samples (2.5 mL) were obtained at 30-min intervals, and a portion (0.5 mL) was injected immediately into a blood gas analyzer (model 158 pH Blood Gas Analyzer, Ciba-Corning, Medfield, MA) for determination of partial pressure of carbon dioxide (pCO_2) and pH. All values were corrected for body temperature. The remaining blood (2.0 mL) was analyzed for plasma lactate (No. 836-UV, Lactate Kit, Sigma Chemical Co., St. Louis, MO). The MABP was also recorded at 30-min intervals on a strip chart recorder.

In Expt. 3, birds were randomly assigned to one of three treatment groups to determine the effect of cyclooxygenase inhibition on MCBF hyperemic response to GSH depletion. The birds were implanted with blood flow probe and a carotid cannula as described above. One group of birds (N = 5) monitored for the 120-min experiment, served as time controls for the blood flow studies. An injection of sesame oil (1 mL/kg body wt, i.p.) in three of these birds did not affect any hemodynamic variable. In the other two groups, after an initial 30-min control period, birds were either injected i.v. with indomethacin (INDO, 5 mg/kg body wt) (N = 7) or phosphate buffer vehicle (PO_4, 1 mL/kg body wt) (N = 9) followed 30 min later by DEM (1 mL/kg body wt, i.p.). Indomethacin and DEM were obtained from the Sigma Chemical Co. Hepatic GSH was ascertained in control, PO_4, and DEM treated birds as described below.

Glutathione analysis. Tissue GSH content was determined by HPLC as described by Farriss and Reed [18]. This method employs perchloric acid precipitation of proteins followed by reaction of iodoacetic acid thiols to form *S*-carboxymethyl derivatives and derivatization of amino groups in the supernatant with 1-fluoro-2,4-dinitrobenzene. Derivatized thiols were separated with an ion-exchange column (3-aminopropyl-spherisorb column, 25 cm × 4.6 mm, 5 μm, Custom Columns, Houston, TX) using an ISCO model 2350 pump, model 2360 gradient programmer, and V_4 variable wavelength detector (ISCO, Lincoln, NE).

Table 1. Effect of diethyl maleate on hepatic and duodenal glutathione (GSH) concentrations

Treatment*	GSH (μmol/g)	
	Liver	Duodenum
Control	4.00 ± 0.27 (6)	5.00 ± 0.51 (6)
DEM	0.53 ± 0.27† (7)	0.83 ± 0.47† (7)

* Hepatic and duodenal tissues were obtained from birds 50 min after i.p. injection (1 mL/kg body wt) of sesame oil (control) or diethyl maleate (DEM). Values are means ± SE of the number of observations in parentheses.
† Significantly different from control ($P < 0.05$).

Statistics. Data were subjected to analysis of variance for unbalanced data using the general linear models procedure of SAS [19]. In Expt. 1, each bird served as its own control with comparisons made to values prior to DEM treatment at time 0. In Expt. 2 and 3, a split plot design in time with treatment as the whole plot and time as the subplot was used for statistical evaluation. The bird-within-treatment error term was used to test the main effect of treatment. Differences between means were determined by Student's *t*-test and considered statistically significant if $P < 0.05$.

RESULTS

The effect of DEM on hepatic and duodenal GSH is presented in Table 1. Fifty minutes after DEM treatment, hepatic and duodenal GSH levels in DEM-treated birds were 13 and 17%, respectively, of that exhibited in controls. Oxidized GSH was not detected in the intestine. In the liver, oxidized GSH was less than 5% of reduced GSH and not affected significantly by DEM (data not shown).

Changes in MCBF, MABP and CVR of birds in Expt. 1 are shown in Fig. 1. The MCBF increased from 8.6 ± 0.4 at time 0 to 10.6 ± 0.4 mL/min/kg body wt 10 min after DEM was injected and remained elevated for the rest of the experiment (Fig. 1A). The increase in MCBF was concomitant with a decrease in CVR (Fig. 1C) and followed by a decline in MABP at 30 min (Fig. 1B).

The effects of DEM on acid–base balance, arterial lactate, and MABP are shown in Table 2. Although lactate was higher in birds 90 min after DEM, there were no differences in arterial pH, pCO_2, or blood bicarbonate at any time between DEM and control groups. At 90 min, arterial pCO_2 was lower in comparison to values at time 0 in the DEM group. During the experiment, both groups of birds exhibited a decrease in blood bicarbonate. MABP also declined during the experiment in both groups of birds, but the decrease was greater in birds that received DEM.

To test the hypothesis that the elevation in MCBF following GSH depletion may occur as a result of increased synthesis of vasodilatory prostanoids, birds in Expt. 3 were randomly assigned to a time control group, or were treated with either indomethacin (INDO) or phosphate buffer vehicle (PO_4) 30 min prior to DEM. Depletion of GSH by DEM was unaf-

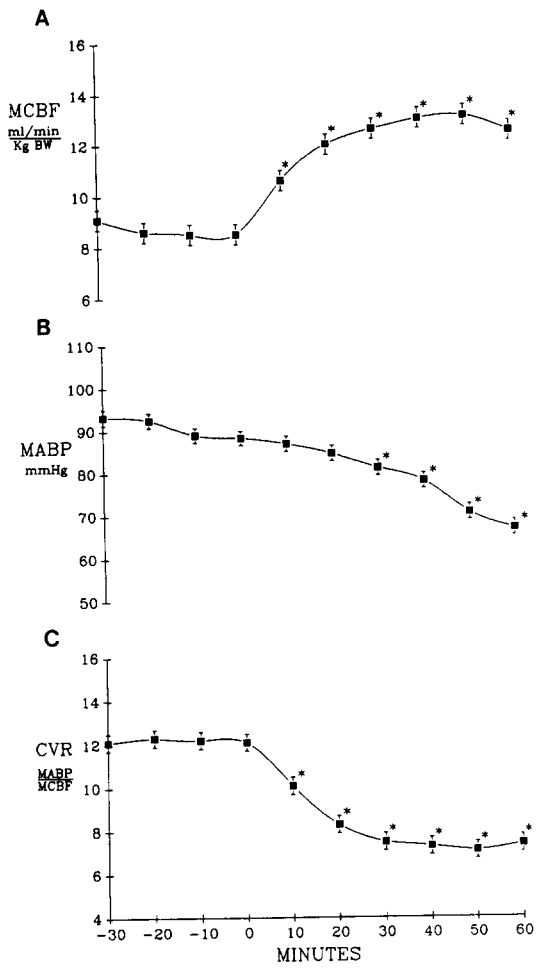

Fig. 1. Effect of diethyl maleate (DEM) on (A) mean celiac blood flow (MCBF, in mL/min/kg body wt), (B) mean arterial blood pressure (MABP, in mm Hg), and (C) celiac vascular resistance (CVR, calculated as MABP/MCBF) in Expt. 1 (N = 10). DEM was injected i.p. at time 0. Values are means ± SE. Key: (*) Significantly different from time 0 ($P < 0.05$).

fected by pretreatment of birds with indomethacin, with hepatic GSH levels for control, PO_4, and INDO treated birds of 2.84 ± 0.27, 0.39 ± 0.16, and 0.40 ± 0.11 μmol/g respectively. While these values were slightly lower, the extent of GSH depletion (to 14% of control) in PO_4 and INDO treated birds was nearly identical to the previous experiment.

The effects of the various treatments on MCBF, MABP, and CVR in birds in Expt. 3 are shown in Fig. 2. In control birds, these hemodynamic variables remained unchanged throughout the experimental time period. It is evident that indomethacin attenuated the rise of MCBF following GSH depletion (Fig. 2A). At the end of the experiment, MCBF was 13.8 ± 0.6, 11.8 ± 0.7, and 10.6 ± 0.8 mL/min/kg body wt in the PO_4, INDO, and control groups respectively. Cyclooxygenase inhibition, however, did not prevent the drop in MABP following DEM (Fig. 2B). After 60 min, DEM treatment had decreased CVR by approximately 40% in PO_4 and INDO treated birds compared to a 10% decrease in the control group (Fig. 2C).

DISCUSSION

To our knowledge, this is the first report of the effect of DEM on tissue GSH content in domestic fowl. The 86–87% decrease of hepatic GSH following DEM treatment with or without indomethacin pretreatment was similar to that reported in mice and rats [1, 3, 5]. Although hepatic tissue generally has the highest concentration of GSH [1], duodenal GSH levels were higher with conditions and methods used in this study.

The elevation of plasma lactate following DEM treatment in this study was similar to observations in rabbits [6] and may result from impairment of lactate metabolism [20]. Although an uncompensated metabolic acidosis was reported in rabbits 60 min after DEM treatment [6], similar disruptions of acid-base balance following DEM treatment apparently do not occur in domestic fowl.

In rabbits, Bill [21] reported that indomethacin (20 mg/kg body wt) decreased splanchnic blood flow

Table 2. Effect of diethyl maleate on mean arterial blood pressure (MABP), arterial lactate, pH, pCO_2, and bicarbonate in domestic fowl*

Treatment	Time (min)	MABP (mm Hg)	Lactate (mmol/L)	pH	pCO_2 (torr)	HCO_3^- (mmol/L)
Control	−30		1.4 ± 0.1	7.45 ± 0.02	38.0 ± 2.6	25.3 ± 0.7
	0	96 ± 4	1.4 ± 0.1	7.45 ± 0.03	39.1 ± 3.5	26.0 ± 0.8
	30	89 ± 5	1.5 ± 0.1	7.45 ± 0.02	36.6 ± 3.5	24.4 ± 1.2
	60	83 ± 6	1.5 ± 0.1	7.46 ± 0.03	33.9 ± 3.1	23.1 ± 0.8
	90	75 ± 5	1.6 ± 0.1	7.47 ± 0.03	32.1 ± 2.8	22.1 ± 0.2†
DEM	−30		1.3 ± 0.2	7.45 ± 0.02	38.0 ± 1.1	24.9 ± 0.9
	0	95 ± 10	1.4 ± 0.1	7.46 ± 0.01	37.0 ± 1.4	25.6 ± 1.1
	30	77 ± 3	1.5 ± 0.1	7.45 ± 0.02	34.3 ± 2.2	23.2 ± 1.9
	60	61 ± 4†‡	2.0 ± 0.3†	7.46 ± 0.02	31.2 ± 3.2	21.4 ± 1.4†
	90	53 ± 7†‡	2.2 ± 0.3†‡	7.48 ± 0.02	28.4 ± 1.8†	20.3 ± 1.7†

* Birds were injected i.p. (1 mL/kg body wt) at time 0 with sesame oil (control) or diethyl maleate (DEM). Abbreviations: MABP, mean arterial blood pressure; pCO_2, partial pressure of carbon dioxide: and HCO_3^-, bicarbonate. Values are means ± SE of four observations.

† Means within a column were significantly different ($P < 0.05$) from within group values at time 0.

‡ DEM mean was significantly different from time control ($P < 0.05$).

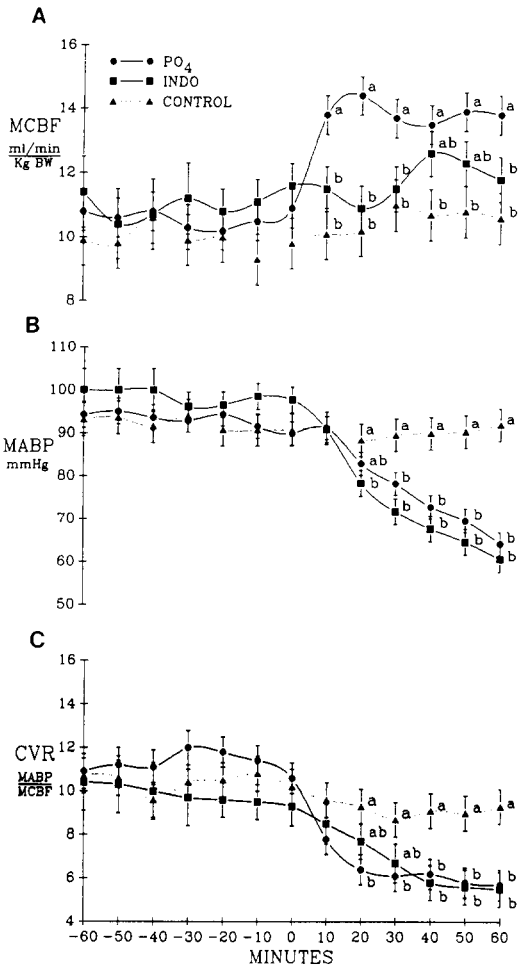

Fig. 2. Changes in (A) mean celiac blood flow (MCBF, in mL/min/kg body wt), (B) mean arterial blood pressure (MABP, in mm Hg), and (C) celiac vascular resistance (CVR, calculated as MABP/MCBF) in time controls (N = 5) and in birds pretreated with indomethacin (INDO, 5 mg/kg body wt (N = 7) or phosphate buffer vehicle (PO_4) (N = 9) in Expt. 3. Indomethacin or phosphate buffer was injected (i.v.) at −30 min followed by diethyl maleate at time 0. Values are means ± SE. Key: (ab) Values with different letters were significantly different ($P < 0.05$).

20 min after administration and suggested that prostaglandins play a key role in the control of resting blood flow in the gastrointestinal circulation. In the present study, MCBF and CVR were unaffected during the 30-min period following indomethacin treatment (Fig. 2). Thus, it appears that either: (1) prostaglandins do not play a significant role in regulating basal vasomotor tone of the celiac artery, or (2) that the level of indomethacin used (5 mg/kg body wt) had no effect on celiac vasomotor tone in domestic fowl.

The attenuation of the MCBF rise following GSH depletion by indomethacin would appear to implicate that increased synthesis of vasodilatory prostaglandins occurred in the intestinal vasculature following GSH depletion. These findings support the hypothesis by Bottje *et al.* [13] that increased hepatic perfusion after DEM treatment in the rat resulted from elevated prostaglandin synthesis. Presumably, cyclooxygenase activity increases with GSH depletion as a consequence of elevated peroxide tone [22, 23]. Several studies have indicated that GSH depletion with buthionine sulfoximine increases prostacyclin synthesis [9, 10, 24]. Since prostacyclin is a potent vasodilator, the elevation of MCBF following DEM may have resulted from increased synthesis of this or another vasodilatory prostanoid.

Buthionine sulfoximine decreases tissue GSH content by inhibiting γ-glutamylcysteine synthase with maximal GSH depletion occurring after 4 hr [1]. While buthionine sulfoximine may be preferred to DEM as a GSH-depleting agent [1], maintaining animals under anesthesia for a 4-hr experimental period could in itself result in deterioration of hemodynamic variables. With this experimental constraint, the rapid depletion of GSH which occurs with DEM may be more suitable for hemodynamic studies.

A significant decrease in MABP was observed in control birds in the acid–base study (Expt. 2), but not in time control birds associated with Expt. 3. The difference between these experiments may be due to blood sampling in Expt. 3 since a small amount of blood loss can reduce blood pressure in birds [16, 17]. Although MABP decreased in control birds in Expt. 3, the MABP drop was greater in DEM-treated birds (Table 2).

The decrease in MABP following DEM treatment in birds in this study differs from that in the rat in which DEM had no effect on blood pressure [25]. The fall in MABP following GSH depletion apparently did not result from increased prostaglandin synthesis since cyclooxygenase inhibition failed to attenuate the fall in blood pressure following DEM treatment (Fig. 2). Although DEM does not appear to affect hepatic function [20, 26] nor does DEM induce liver damage *in vivo* [6], the use of DEM has been criticized since it may produce non-specific toxicities [1]. Based on the results of the present study, the exact mode of action by which DEM or associated depletion of GSH mediates its adverse effect on blood pressure regulation in anesthetized domestic fowl is not apparent, but may result from a non-specific toxic action, e.g. on central blood pressure regulation.

In summary, DEM-mediated depletion of hepatic and duodenal GSH was associated with elevated plasma lactate and celiac blood flow and decreased mean arterial pressure and celiac vascular resistance in domestic fowl. GSH depletion by DEM had no effect on the bicarbonate buffer system. Pretreatment of birds with a cyclooxygenase inhibitor, indomethacin, had no effect on the response of blood pressure or celiac vascular resistance to glutathione depletion. However, since the rise in celiac blood flow was attenuated by indomethacin, this vascular response could be mediated by enhanced synthesis of vasodilatory prostaglandins in the intestinal circulation following glutathione depletion. Additional studies in which circulating levels of arachidonic acid metabolites are measured would be required in order to fully evaluate the relationships between GSH

depletion and prostanoid synthesis in the domestic fowl.

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