Regulation of Endothelial Cell Prostaglandin Synthesis by Glutathione*

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The major eicosanoid products of vascular endothelium, prostacyclin (PGI2) and PGE2, play important roles in vascular homeostasis by exerting vasodilatory, anti-inflammatory, and anti-thrombotic influences and by regulating permeability and cholesterol metabolism (1-4). Recent studies have demonstrated prostaglandin-mediated changes in organ blood flow under conditions in which the supply of reduced glutathione (GSH) is limited (5-7). Alterations in cellular GSH, which may occur during oxidant stress and xenobiotic metabolism, might thereby alter vascular functions of the endothelium. For these reasons, we wished to investigate the role of GSH in regulating prostaglandin synthesis in vascular endothelium.

In vascular tissue, prostaglandin production is regulated both by the availability of unesterified arachidonic acid, which is released from phospholipid stores upon activation of phospholipase A2, and by the activities of synthetic enzymes. These include prostaglandin H synthase (PGHS), which catalyzes the conversion of arachidonic acid to prostaglandin H2 (PGH2), prostacyclin synthase, and PGE isomerase, which catalyze the conversion of PGH2 to prostacyclin and PGE2, respectively. Numerous studies in vitro using microsomes, purified enzyme preparations, and tissue homogenates have shown that activities of prostaglandin synthetic enzymes are modulated by peroxide levels (8-13). While the cyclooxygenase component of PGHS requires low levels of hydroperoxides for activation and continued catalysis, higher levels of hydroperoxides are inhibitory (11, 14). Prostacyclin synthase is inactivated by lipophilic hydroperoxides in a similar fashion (9). The activity of PGHS is also limited by autoinactivation, due to the formation of a reactive intermediate (15, 16).

Numerous studies have demonstrated effects of hydroperoxide exposure on prostaglandin production in intact cells. Biphasic responses have been observed in lung fibroblasts (17), mesangial cells (18), and vascular tissue (19, 20). Low levels of peroxides stimulate prostaglandin production by activating PGHS and perhaps by increasing the availability of arachidonic acid (17, 21-24). Higher levels irreversibly inactivate PGHS (17, 25, 26). Previous studies from our laboratory have shown that low levels of lipophilic hydroperoxides are capable of stimulating PGHS while at the same time inhibiting prostacyclin synthase (27). In addition, we have demonstrated a dose-dependent inhibition of PGHS by sublethal doses of hydrogen peroxide in vascular endothelial cells with similar doses exerting no inhibitory effect on prostacyclin synthase (26).

Studies in vitro have shown that GSH also impacts on prostaglandin production. First, GSH is a necessary cofactor in the peroxide-removing reaction catalyzed by glutathione peroxidase. The addition of glutathione peroxidase and GSH to purified PGHS prior to substrate addition increased the lag period for cyclooxygenase activation, while the addition of glutathione peroxidase and GSH during catalysis completely inhibited the cyclooxygenase reaction (11). Both effects have been attributed to removal of “activator” hydroperoxides by glutathione peroxidase. Secondly, GSH can serve as a reducing cofactor for the peroxidase component of PGHS, resulting in the formation of a glutathionyl radical (28). Finally, GSH is required as a cofactor for PGE isomerase, the enzyme which converts PGH2 to PGE2 (29).

Few studies, however, have addressed the role of GSH in prostaglandin production by intact cells. Rouzer et. al. (30) showed that GSH-depletion in mouse peritoneal macrophages...
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led to decreased synthesis of PGE₂, the major prostaglandin product, although the total amount of prostaglandins produced remained the same. Synthesis of PGE₂ was reduced in rat alveolar macrophages exposed to sulphydryl-reactant agents such as N-ethylmaleimide and acrolein concomitant with an increase in total prostaglandin synthesis (22, 31). Whether the latter effect was due to increased availability of arachidonic acid or to altered activities of prostaglandin synthetic enzymes was not determined.

For these reasons, we hypothesized that the capacity of GSH to scavenge peroxides may be important in regulating activities of PGHS and prostacyclin synthase in vascular endothelium. The aim of the present study was to examine the regulation of prostaglandin synthesis by GSH in the absence and presence of exogenous peroxides using intact vascular endothelial cells. Our studies demonstrate that GSH depletion enhances prostaglandin synthesis in the absence of exogenous hydroperoxides, while increasing the sensitivity of prostaglandin synthesis to inhibition by exogenous hydroperoxide.

EXPERIMENTAL PROCEDURES

Materials—Tissue culture supplies, including Dulbecco's modified Eagle's medium, Hanks' balanced salt solution, antibiotic/antimycotic (10,000 units of penicillin, 10,000 μg of streptomycin, and 25 μg of fungizone/ml), and trypsin-EDTA were obtained from Gibco. Fetal bovine serum was from HyClone (Logan, UT), and plasticware was from Nunc (PQG Scientific, Gaithersburg, MD). Radiolabels were obtained from Du Pont-New England Nuclear (5,6,8,9, 11,12,14,15-³H)arachidonic acid) and from Merck, Sharp and Dohme ([3,3,4,4-³H]keto-prostaglandin F₁, and [3,3,4,4-³H]prostaglandin E₁). [³H]PGH₂ was synthesized from [³H]arachidonic acid using microsomes from ram seminal vesicles (32). Derivatizing reagents, including l-chloro-2,4-dinitrobenzene and acetic anhydride, and methoxamine hydrochloride were from Pierce Chemical Co. PGH₂ was supplied by Cayman Chemical Co. (Ann Arbor, MI) and arachidonic acid by Calbiochem (San Diego, CA). All other chemicals and reagents were obtained from Sigma. All solvents used were of high performance liquid chromatography grade and derivatizing reagents were of the highest quality available.

Cell Culture—Endothelial cells were isolated from freshly obtained porcine aortas by collagenase treatment as previously described (33, 34). Cells were plated at a density of 400,000 cells/flask in 25-cm² tissue culture flasks in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% antibiotic/antimycotic and incubated at 37 °C in 10% CO₂/90% air. Primary and subsequent cultures were treated with 0.1% trypsin-EDTA and split 1:4 for subculture. Cultured endothelial cells were polygonal in shape, demonstrated contact inhibition, and stained positively for Factor VIII related antigen. Confluent cultures in the second or third passage were used for study. Except where indicated, all experimental incubations were carried out in Hanks' balanced salt solution containing 10 mM Hepes (HHBSS) in room air at 37 °C following rinsing of the cells three to four times.

Glutathione Depletion—Treatment of cells with buthionine sulfoximine (BSO) was carried out in Dulbecco's modified Eagle's medium containing 1% fetal bovine serum and 1% antibiotic/antimycotic for up to 24 h. Diethylmaleate (DEM) and 1-chloro-2,4-dinitrobenzene (CDNB) were dissolved in dimethyl sulfoxide (final concentration 1%) and incubated with cells in HHBSS for 30–60 min. Control groups were incubated with 1% dimethyl sulfoxide as vehicle control. For analysis of cellular GSH, cells were rinsed three times with HHBSS and residual buffer removed by aspiration. Cells were scraped into 1.5 ml of ice-cold 1 M perchloric acid containing 2 mM EDTA and extracted for 30 min at 4 °C. Following centrifugation at 600 × g, the acid extract was neutralized with 4 M KOH containing 0.6 M MOPS. Samples were then centrifuged to remove the potassium perchlorate precipitate and assayed immediately. GSH content was determined by spectrophotometric methods using the GSH reductase-linked assay of Tietze (35). Standard curves were prepared on the day of analysis using 0.1–1.0 μM GSSG. GSH content was expressed in terms of "GSH equivalents." Prostaglandin Analysis—Both basal production of prostaglandin metabolites and prostaglandin synthetic capacity were determined by analysis of media obtained from cells after 3 min of incubation in HHBSS (or other time periods as indicated). Prostacyclin was measured as its stable breakdown product 6-keto-PGF₁α. Synthetic capacity was determined by subtracting basal prostaglandin production from production in the presence of saturating levels of exogenous prostanoid (36) (i.e. 20 μM arachidonic acid). For these experiments, the total amount of metabolites produced from arachidonic acid represented PGHS activity. Activity of prostacyclin synthase activity was determined by measurement of PGI₂ production from PGH₂.

Measurement of PGH₁ and PGF₂α was accomplished by gas chromatography-mass spectrometry following the addition of deuterated internal standards and derivatization of samples as previously described (34). Briefly, 600–800 ng of deuterated internal standards were added to cell culture media. Following acidification to pH 3 with formic acid, samples were extracted with ethyl acetate, dried under N₂, redissolved in 0.1 ml of methanol, and treated with 0.2 ml of ethereal diazomethane for 5 min to form the methyl ester. After evaporation under N₂, samples were reacted overnight with a saturated solution of methoxyamine hydrochloride in pyridine to form the methoxime derivative. After extraction with diethyl ether, samples were reacted with 20 μl of N,O-bis-[trimethylsilyl] trifluoroacetamide for 2 h, evaporated under N₂, and redissolved in hexane. Analysis was accomplished by selected ion monitoring using a gas chromograph-mass spectrometer (model 5995B, Hewlett-Packard, Palo Alto, CA).

Measurement of Microsomal PGHS and Prostacyclin Synthase Activity—Direct effects of GSH-depleting agents on PGHS activity were measured in ram seminal vesicle microsomes by an oxygen consumption assay. Microsomes were prepared by homogenizing pieces of frozen seminal vesicles in ice-cold 20 mM Tris-HCl containing 5 mM EDTA and 5 mM diethyldithiocarbamate, pH 8.0. Following centrifugation at 10,000 × g for 15 min at 4 °C, supernatant was filtered through four layers of cheesecloth and subjected to centrifugation at 100,000 × g for 60 min at 4 °C. The resulting pellet was resuspended in hand-held homogenizer in the same buffer containing 1% Tween 20 following sonication (Virsionic Cell Disruptor model 18-850, Virsonics, Inc., Gardiner, NY) two times for 15 s at 35% capacity, samples were subjected to centrifugation at 100,000 × g for 60 min at 4 °C. The resulting supernatant was kept at 4 °C until analysis. Protein content of the solubilized microsomes was determined by the method of Lowry et al. (37). The oxygen consumption assay was carried out at 37 °C in 0.2 M Tris-HCl containing 1 μM hematin and 0.5 mM phenol, pH 8.0, using a YSI model 5300 Biological Oxygen Monitor and model 5775 Oxygen Probe (YSI Co., Yellow Springs, OH). Aliquots of solubilized microsomes were incubated for 5 min in the assay system in the presence and absence of GSH-depleting agent by vehicle prior to the addition of 50 μM arachidonic acid. Prostanoid activity was calculated from the initial velocity of the reaction in terms of nanomoles of O₂ consumed/minute/milligram of protein.

To test the direct effects of GSH-depleting agents on prostacyclin synthesis, production of 6-keto-PGF₁α from PGH₂ was measured in microsomes from porcine aorta. Porcine aorta were rinsed and homogenized in 50 mM Tris-HCl buffer containing 1 mM EDTA, pH 7.4, and centrifuged as described above. Microsomes were then incubated for 5 min at 25 °C in the presence or absence of treatment followed by the addition of 1.2 μg/ml of [³H]PGH₂ (5 × 10⁶ cpm/μg). After 3 min, the reaction was stopped with cold acetone, and samples were extracted with hexane and chloroform as previously described (38). Prostaglandin products were separated by thin layer chromatography using the organic phase of ethyl acetate/isooctane/acetic acid/H₂O (110:40:20:100).

Cytotoxicity—Cytotoxicity of GSH-depleting agents and hydroperoxides was determined by quantitating the amount of lactate dehydrogenase released from cells either during incubation with the agents or during a subsequent incubation in HHBSS. The amount of lactate dehydrogenase released was compared with the amount contained in cell lysates prepared by solubilizing and sonication in HHBSS containing 0.05% Triton X-100. Neither GSH-depleting agents nor hydroperoxides were found to inhibit lactate dehydrogenase since total (lactate plus released) lactate dehydrogenase was not decreased compared with controls. Lactate dehydrogenase was assayed by spectrophotometric methods (39).

Statistics—Data were analyzed by analysis of variance using PC SAS (SAS Institute, Cary, NC). Multiple comparisons were made (40) with the significance level set at p < 0.05.
RESULTS

Treatment of endothelial cells with 1 mM BSO, a specific inhibitor of GSH synthesis (41), resulted in a time-dependent decrease in cellular GSH content by 85% (Fig. 1A). A separate experiment demonstrated reduction of cellular GSH levels from 56.9 ± 2.8 nmol/mg protein to 12.0 ± 1.8 nmol/mg protein using 0.1 mM BSO and to 8.8 ± 0.8 nmol/mg protein using 1.0 mM BSO over a 24-h period. The turnover rate of GSH in endothelial cells was slow, with a half-life of 6.3 h as determined by log plot of data from Fig. 1A. No cytotoxicity or change in cellular protein levels was associated with BSO treatment.

Total prostaglandin synthesis was significantly higher in cells treated with 1 mM BSO for 24 h (Fig. 1B). Since this change was measured in the presence of saturating levels of substrate (36), it reflected a change in synthetic enzyme activity and not merely an alteration in substrate availability. Neither basal prostaglandin production nor PGE2 production was altered by BSO treatment. Hence, the increase in synthetic capacity, which was measured as the difference between basal and arachidonate-stimulated cells, was due solely to increased PGI2 production. In seven separate experiments, the average increase in PGI2 synthetic capacity was 36 ± 8%.

Since depletion of GSH may alter the kinetics for initiation of PGHS catalysis, we examined the time course of PGI2 and PGE2 production from exogenously added arachidonic acid over a 10-min period. PGI2 production by BSO-treated cells was significantly elevated over controls at all time points studied (Fig. 2A). When corrected for basal prostaglandin production, PGI2 synthetic capacity over 10 min was 369 ±

37 ng in BSO-treated cells compared with 202 ± 20 ng in controls. BSO treatment had no statistically significant overall effect on PGE2 production (Fig. 2B), although PGE2 synthetic capacity was somewhat higher in BSO-treated cells compared with controls at 10 min (56.6 ± 5.3 ng compared with 36.9 ± 4.5 ng). It is also apparent that activation of prostaglandin synthesis occurred more rapidly in BSO-treated cells. When data from Fig. 2A were converted to express the rate of PGI2 production, typical activation-inactivation kinetics for prostaglandin synthesis was seen. Importantly, the BSO-treated group exhibited a higher rate of synthesis compared with controls (Fig. 3). These data indicate that BSO
Prostaglandin production was also measured in endothelial cells treated with agents which deplete GSH over a shorter time course than BSO. This was done in order to determine whether the effects of BSO were secondary to long term GSH depletion rather than a direct result of GSH depletion. DEM and CDNB decrease cellular GSH levels by glutathione-S-transferase-catalyzed conjugation with GSH (42). N-Ethylmaleimide (NEM) acts by alkylating thiols including GSH. Dose-response relationships for GSH depletion by these agents are shown in Fig. 4. Treatment of cells with 1 mM DEM for 60 min and 5 μM CDNB for 30 min were not associated with any cytotoxicity (Table I). However, treatment of cells with NEM caused significant cell damage and could not be used in these protocols (Table I).

Prostaglandin production in CDNB and DEM-treated cells is shown in Fig. 5. Synthesis of PGI₂ from exogenous substrate was significantly enhanced above control levels by 27 and 149% in cells treated with 5 μM CDNB for 30 min and cells treated with 1 mM DEM for 60 min, respectively. DEM and CDNB had no effect on basal prostaglandin production and failed to alter PGE₂ synthetic capacity at doses associated with GSH depletion. Since DEM and CDNB enhanced prostaglandin synthesis, it is unlikely that changes in prostaglandin metabolism were secondary effects resulting from long term treatment with BSO. However, it should be noted that DEM had a much greater effect on PGI₂ production from arachidonate than BSO (Fig. 1B) despite the fact that GSH levels were reduced to a smaller degree in DEM-treated cells compared with BSO-treated cells (Fig. 1A). Thus, DEM probably had other effects on PGHS which were unrelated to GSH depletion (43).

Alterations in GSH levels may regulate prostaglandin syn-

![Figure 4](image1.png)

**Fig. 4.** Dose-response of DEM (panel A), CDNB (panel B), and NEM (panel C) on cellular glutathione content. Cells were treated with DEM for 60 min, CDNB for 30 min, and NEM for 60 min using 1% dimethyl sulfoxide as vehicle. Controls were also treated with 1% dimethyl sulfoxide. Values are mean ± S.E. for three observations/group. Significant differences compared with controls (p < 0.05) are denoted by *.

![Figure 5](image2.png)

**Fig. 5.** Dose response of DEM (panel A) and CDNB (panel B) on prostaglandin synthetic capacity 3 min following the addition of 20 μM arachidonic acid. PGI₂ was measured as its stable breakdown product 6-keto-PGF₁α. Cells were treated with 1 mM DEM for 60 min and 5 μM CDNB for 30 min. Values are mean ± S.E. for three observations/group. Significant differences compared with controls (p < 0.05) are denoted by *. Basal prostaglandin production over 3 min in controls was 46.8 ± 6.3 ng for PGI₂ and 28.1 ± 0.9 ng for PGE₂.

5. Synthesis of PGI₂ from exogenous substrate was significantly enhanced above control levels by 27 and 149% in cells treated with 5 μM CDNB for 30 min and cells treated with 1 mM DEM for 60 min, respectively. DEM and CDNB had no effect on basal prostaglandin production and failed to alter PGE₂ synthetic capacity at doses associated with GSH depletion. Since DEM and CDNB enhanced prostaglandin synthesis, it is unlikely that changes in prostaglandin metabolism were secondary effects resulting from long term treatment with BSO. However, it should be noted that DEM had a much greater effect on PGI₂ production from arachidonate than BSO (Fig. 1B) despite the fact that GSH levels were reduced to a smaller degree in DEM-treated cells compared with BSO-treated cells (Fig. 1A). Thus, DEM probably had other effects on PGHS which were unrelated to GSH depletion (43).
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Partially purified PGHS from ram seminal vesicle microsomes was preincubated for 5 min with drug prior to addition of 50 μM arachidonate to initiate the reaction. DEM and CDNB were dissolved in dimethyl sulfoxide (1% final concentration). Data are expressed as means ± S.E. for n values (in parentheses).

| Treatment        | nmol O consumed/min/mg protein |
|------------------|-------------------------------|
| A. Control       | 290 ± 8 (17)                  |
| Dimethyl sulfoxide| 303 ± 16 (6)                  |
| DEM 1 mM         | 301 ± 8 (6)                   |
| CDNB 5 μM        | 277 ± 8 (6)                   |

B. Control       | 547 ± 20 (4)                  |
| BSO 1 mM        | 542 ± 23 (4)                  |

Prostacyclin synthase assay

Porcine aortic microsomes (8 mg protein/ml) were preincubated for 5 min with drug prior to addition of 2.5 μg of [3H]PGH₂ to initiate the reaction. DEM and CDNB were dissolved in dimethyl sulfoxide (1% final concentration). Data are expressed as means ± S.E. (n = 3).

| Treatment        | 6-keto-PGF₁α cpm |
|------------------|------------------|
| A. Control       | 8,830 ± 1,190    |
| BSO 1 mM         | 8,750 ± 1,620    |
| GSSG 1 mM        | 9,210 ± 1,120    |
| GSH 1 mM         | 6,420 ± 580      |

B. Dimethyl sulfoxide (1%) | 14,500 ± 2,190 |
| DEM 1 mM          | 11,100 ± 2,450  |
| CDNB 5 μM         | 9,060 ± 3,700   |

Fig. 7. Effect of BSO and H₂O₂ on PG₁₂ synthetic capacity 3 min following the addition of 20 μM arachidonic acid. PG₁₂ was measured as its stable breakdown product 6-keto-PGF₁α. Cells were treated with 1 mM BSO for 24 h and H₂O₂ for 10 min. Values are mean ± S.E. for five observations/group. Significant differences compared with corresponding controls (p < 0.05) are denoted by *. Significant differences compared with the non-BSO, non-H₂O₂ treated group are denoted by **. Basal PG₁₂ production over 3 min was 19.9 ± 0.9 ng in control cells and 19.7 ± 1.3 ng in BSO-treated cells.

Fig. 6. Effect of treatment with 1 mM BSO for 24 h (panel A) and 1 mM DEM for 60 min (panel B) on PG₁₂ production 3 min following the addition of 3 μM PGH₂. PG₁₂ was measured as its stable breakdown product 6-keto-PGF₁α. Values are mean ± S.E. for four observations/group. Significant differences compared with non-BSO-treated controls (p < 0.05) are denoted by *. Basal PG₁₂ production was not significantly different in treated groups than controls in either experiment.

Since GSH depletion may impact the maintenance of cellular peroxide tone during oxidant stress, we also examined prostaglandin synthetic capacity in BSO-treated cells which were exposed to exogenous hydroperoxides. Previous experiments from our laboratory demonstrated dose-dependent inhibition of PGHS in porcine aortic endothelial cells by H₂O₂ with an IC₅₀ of 35 μM (26). Prostacyclin synthase, however, was unaffected by 50 μM H₂O₂ (26). Fig. 7 demonstrates PG₁₂ synthetic capacity following the addition of arachidonic acid in control and BSO-treated cells exposed to 0–50 μM H₂O₂ for 10 min. These exposures to H₂O₂ were not associated with
cytotoxicity in control or BSO-treated cells (Table IV). In cells not treated with BSO, 5 μM H₂O₂ significantly increased prostacyclin synthetic capacity by 25%, while 25–50 μM H₂O₂ inhibited PGI₂ synthetic capacity by 30% and 75%, respectively. BSO treatment was found to enhance PGI₂ synthetic capacity by 42% in the absence of H₂O₂. This degree of enhancement was decreased to 22% in the presence of 25 μM H₂O₂. Furthermore, BSO-treated cells which were exposed to 50 μM H₂O₂ synthesized 96% less PGI₂ than cells exposed to 50 μM H₂O₂ alone. These data clearly show that GSH depletion led to an enhanced susceptibility of prostaglandin production to inhibition by 50 μM H₂O₂.

**DISCUSSION**

The present study demonstrates that GSH regulates prostaglandin synthesis in porcine aortic endothelium. Using three agents which reduce cellular GSH by different mechanisms, we have shown that PGI₂ synthesis was enhanced significantly by GSH depletion. Since none of the GSH-depleting agents directly enhanced microsomal PGH₂ or prostacyclin synthase activity, GSH depletion itself appears responsible for the enhanced prostaglandin synthesis.

Our studies have shown that the initial reaction rate, as well as the extent, of PGI₂ synthesis was increased in GSH-depleted cells compared with controls. Since hydroperoxides are known to activate PGH₂, it is likely that the mechanism responsible for this effect was the increased production of "activator hydroperoxides" in GSH-depleted cells. This hypothesis is supported by the fact that altered prostaglandin production in GSH-depleted cells was observed only in the presence of exogenous arachidonate. Under these conditions the turnover of PGHS rapidly increases due to the production of activator hydroperoxides. Thus, PGHS would be particularly sensitive to increased intracellular hydroperoxide levels during rapid turnover. The sensitivity of endothelial cell PGHS to low levels of hydroperoxides was also demonstrated by the 25% increase in prostaglandin synthetic capacity observed in cells exposed to 5 μM H₂O₂ and stimulated with exogenous arachidonate (Fig. 7). Of further interest is our finding that the velocity of PGI₂ synthesis peaked at approximately the same time in control cells and GSH-depleted cells (Fig. 3). Since the velocity of prostaglandin synthesis reflects the net activity of PGHS, this result indicates that GSH-depletion led to more rapid inactivation, as well as more rapid activation, of PGHS compared with controls.

We also found that GSH depletion resulted in increased activity of prostacyclin synthase. This effect was probably not related to altered levels of activator hydroperoxides since prostacyclin synthase is not activated by hydroperoxides. Our results failed to demonstrate altered PGE isomerase activity in GSH-depleted cells. This was unexpected given the known requirement of PGE isomerase for GSH as cofactor (29). Although it is possible that the 85% reduction in intracellular GSH achieved in BSO-treated cells was insufficient to inhibit PGE isomerase, it seems unlikely given the ability of BSO to alter PGE₂ production in macrophages (30). BSO treatment also altered leukotriene C₄ production in macrophages and lung tissue (30, 44) presumably due to decreased GSH-S-transferase activity. Findings of the present study may in fact be due to the combined effects of stimulating PGHS and inhibiting PGE isomerase as a result of GSH depletion. Under these conditions, a partial decrease in PGE isomerase activity due to cofactor limitation might be compensated for by an increase in substrate due to increased PGHS activity. Additionally, findings of the present study might be explained by a concerted increase in the activities of PGHS and prostacyclin synthase. Close coupling between these two enzymes might occur such that the majority of the PGH₂ produced was rapidly metabolized by prostacyclin synthase. This is an important concept since others have suggested that PGHS activity is the primary determinant of PGI₂ synthesis while prostacyclin synthase activity is present in excess. In any case, our study is the first to report GSH-dependent modulation of PGHS and prostacyclin synthase in intact cells.

However, modulation of PGHS and prostacyclin synthase by thiol agents including GSH has been observed in isolated systems. In rat seminal vesicle microsomes, a 2-min preincubation with 0.5–1.0 mM GSH maximally decreased oxygen consumption by 60% (IC₅₀ = 250 μM) (28). Prostaglandin production in platelet microsomes and in isolated aortas was also inhibited by 50% or more in the presence of 1–5 mM GSH (19, 45). These effects on PGHS activity were probably due to enhanced GSH peroxidase-dependent removal of activator hydroperoxides in the presence of exogenous GSH. It should be noted that GSH levels in homogenates are at least two orders of magnitude lower than in vivo (13). Isolated tissues would also be expected to have lower levels of GSH. Conversely, glutathione peroxidase-mediated inhibition of prostaglandin synthesis in bovine vesicular gland homogenates and platelet lysates was much less effective in the absence of exogenous GSH. (8, 46). Other thiol agents, dithiothreitol and cysteine, inhibited prostaglandin production in vitro (16, 19). These actions were likely due to non-enzymatic reduction of hydroperoxides in the reaction mixture (8). Thiol agents also altered the activity of prostacyclin synthase in microsomes (47, 48). Physiologically relevant concentrations of GSH, as well as dithiothreitol, enhanced the production of PGI₂ from PGH₂. The mechanism responsible for these effects, which do not agree with our findings in intact cells, was not investigated.

Our hypothesis that GSH depletion alters endothelial cell prostaglandin synthesis as a consequence of higher levels of activator hydroperoxides presumes that the degree of GSH depletion achieved in our studies was sufficient to limit the activity of glutathione peroxidase. This is difficult to determine since kinetic studies of glutathione peroxidase in vitro have demonstrated that the velocity of the glutathione peroxidase-catalyzed reaction is dependent on concentrations of both peroxide and GSH (49). However, it has been estimated that a 90% reduction of cellular GSH levels leads to decreased velocity of glutathione peroxidase-catalyzed reduction of hydroperoxides (50). Since we achieved similar levels of GSH depletion in this study, it is likely that the activity of glutathione peroxidase was limited.
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thione peroxidase was decreased in BSO-treated cells. Our findings of enhanced prostaglandin synthesis in GSH-depleted cells are consistent with in vitro findings which suggest that GSH/glutathione peroxidase may be an important endogenous inhibitor of prostaglandin biosynthesis in vivo (8, 13).

Direct thiol-disulfide regulation of enzymatic activity may also play a role in mediating the increased prostaglandin synthesis observed in GSH-depleted cells. This may explain the effect of GSH depletion on prostacyclin synthase, which is not sensitive to activator hydroperoxides. Although the primary sequence of prostacyclin synthase contains 4 cysteines, it is not known whether they form disulfide bridges which might be regulated by thiol-disulfide status (51).

We also studied the effects of GSH depletion on prostaglandin synthesis in porcine aortic endothelial cells exposed to sublethal concentrations of exogenous hydroperoxides. These experiments were carried out because GSH depletion is likely to impact on the maintenance of cellular peroxide concentrations during cellular oxidant stress. Although PGHS requires low levels of activator hydroperoxides for catalysis, higher levels have been shown to cause enzymatic inactivation in vitro and in vivo (11, 14, 17–19, 25, 26). The mechanism for this inactivation has not been elucidated but appears to be different than enzyme autoinactivation (11) and may involve the hydroperoxidase function of the enzyme (11, 52). In vitro studies have shown that hydroperoxide-mediated inactivation is decreased in the presence of reducing cofactors for the hydroperoxidase moiety (14, 52). Although physiological concentrations of GSH can act as reducing cofactor for PGHS in microsomes with the subsequent formation of glutathionyl radical (28), the reducing potential of GSH is low compared to endogenous compounds such as urate and ascorbic acid (14). Hence, GSH is not likely to function as an endogenous substrate for the hydroperoxidase in vivo. Much better established is the role of GSH as reducing cofactor for glutathione peroxidase, which is the major pathway for hydroperoxide metabolism in cultured endothelium (53).

Results of the present study demonstrated a biphasic effect of H2O2, with 5 μM stimulating prostaglandin synthesis and 25–50 μM exerting an inhibitory effect. Since endothelial cell prostacyclin synthase is not sensitive to H2O2 at these concentrations (26), these effects are likely mediated through PGHS. In addition, prostaglandin production was enhanced by BSO treatment even in the presence of 5–10 μM H2O2. However, BSO treatment resulted in a dramatic inhibition of prostaglandin synthesis by 50 μM H2O2. These findings suggest that the ability of peroxide-metabolizing systems to reduce cellular peroxide concentrations below levels which cause PGHS inactivation is limited in GSH-depleted cells. This probably reflects impaired glutathione peroxidase activity in GSH-depleted cells which were exposed to exogenous peroxide. Additionally, it is possible that prostacyclin synthase was inactivated in GSH-depleted cells exposed to 50 μM H2O2 resulting from a decreased ability of glutathione peroxidase to metabolize endogenous lipid hydroperoxides.

In summary, GSH depletion was found to alter prostaglandin production in the porcine aortic endothelial cell. Under normal levels of endogenous hydroperoxides and low levels of exogenous hydroperoxides, lowering cellular GSH levels by 85% results in increased PG12 synthesis. However at higher, but still sublethal levels of exogenous hydroperoxides, GSH-depleted cells were more susceptible to inhibition of prostaglandin production. These findings indicate a complex balance between stimulatory and inhibitory influences on enzymes responsible for prostaglandin synthesis. Changes in prostaglandin-dependent vascular functions would thus be expected during xenobiologic metabolism and oxidant stress, conditions which are known to decrease cellular GSH levels. Recent studies have demonstrated thiol-dependent changes in organ blood flow. Increased hepatic and celiac blood flow was observed following GSH depletion using DEM (5, 6). This vasodilatory effect was reversed by partial restoration of GSH levels and was prevented by pretreatment with indomethacin, an inhibitor of PGHS (5). These findings are consistent with our findings of increased production of the potent vasodilator PGI2 following GSH depletion of vascular endothelial cells. In another study, peroxide-mediated pulmonary vasoinconstriction was enhanced in lungs treated with agents which limit the availability of GSH (7). These studies agree with our results showing enhanced susceptibility of PGHS to peroxide-mediated inhibition in GSH-depleted endothelial cells. Thus, cellular GSH levels, as well as peroxide concentrations, may influence vascular function by regulating prostaglandin synthesis in endothelium.

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