Biochemical Pharmacology, Vol. 44, No. 8, pp. 1597–1602, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

INHIBITORY EFFECT OF AN INTRACELLULAR GLUTATHIONE ON Δ^{12}-PROSTAGLANDIN J_2-INDUCED PROTEIN SYNTHESES IN PORCINE AORTIC ENDOTHELIAL CELLS

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(*Received* 8 *April* 1992; *accepted* 25 *June* 1992)

Abstract—Δ^{12}-Prostaglandin (PG) J_2 caused porcine aortic endothelial cells to synthesize a 31,000-dalton heme oxygenase and a 67,000-dalton protein (p67). Treatment of the cells with buthionine sulfoximine (BSO), an inhibitor of glutathione (GSH) synthesis, depleted intracellular GSH, and enhanced the induction of heme oxygenase and p67 syntheses by Δ^{12}-PGJ_2. In contrast, treatment with GSH increased the intracellular GSH level and reduced the induction. There was a reciprocal relationship between the level of intracellular GSH, and that of the induction of heme oxygenase and p67 syntheses by Δ^{12}-PGJ_2. An increase in the intracellular GSH level caused an increase in the ethyl acetate-unextractable form of Δ^{12}-PGJ_2 in the cytosol, but suppressed the accumulation of Δ^{12}-PGJ_2 in the nuclei. Furthermore, GSH strongly inhibited the *in vitro* binding of Δ^{12}-PGJ_2 to isolated nuclei, which is *N*-ethylmaleimide sensitive. Moreover, the induction of heme oxygenase and p67 syntheses by the thiol-reactive agents arsenite and diethylmaleate was also inhibited by GSH treatment and enhanced by BSO treatment. These results demonstrate that intracellular GSH suppresses Δ^{12}-PGJ_2-induced heme oxygenase and p67 syntheses by inhibiting the binding of Δ^{12}-PGJ_2 to nuclei.

9-Deoxy-$\Delta^{9,12}$-13,14-dihydro-prostaglandin D_2 (Δ^{12}-PGJ_2)†, one of the cyclopentenone prostaglandins and the ultimate metabolite of PGD_2 [1], is a potent inducer of growth inhibition for a variety of cultured cells and of cell differentiation in some cell lines [2, 3]. It is now known that Δ^{12}-PGJ_2 is actively transported into cells by a specific carrier and accumulates in the nuclei, where it binds to nuclear proteins [4–6]. Concerning Δ^{12}-PGJ_2-induced growth inhibition, the inhibitory effect of Δ^{12}-PGJ_2 involves the syntheses of specific proteins, especially the induction of 68-kDa proteins, which have been identified as members of the 70-kDa heat shock protein group [7]. We recently found that Δ^{12}-PGJ_2 preferentially induces the synthesis of a 31-kDa protein (p31) as well as a 67-kDa heat shock protein (p67) in porcine aortic endothelial cells (PAEC) [8], and that the Δ^{12}-PGJ_2-induced p31 was heme oxygenase [9].

A characteristic of cyclopentenone prostaglandins is that they have α, β-unsaturated ketones, and α, β-unsaturated carbonyls are very susceptible to nucleophilic addition reactions with thiols such as reduced glutathione (GSH), the most abundant nonprotein thiol *in vivo* [10]. Intracellular GSH plays a significant role as a potent regulator of the oxidation–reduction system. Thus, GSH serves as a reductant in peroxide metabolism, and as a nucleophile in the formation of various drug conjugates, leading to regulation of the action of the drugs and their metabolism [11]. Recently, it was reported that Δ^{12}-PGJ_2 readily conjugates with GSH *in vitro*, and Δ^{12}-PGJ_2 was recovered in a cell lysate primarily as a GSH conjugate [12, 13]. Furthermore, we revealed that the thiol reactive agents arsenite and diethylmaleate also induced the same p31 and p67 syntheses, indicating that the Δ^{12}-PGJ_2-induced heme oxygenase and p67 syntheses are primarily regulated by thiol-reactive agents [8]. From this knowledge it is inferred that intracellular GSH regulates the biological actions of Δ^{12}-PGJ_2. The purpose of the present study was to examine the effect of intracellular GSH on Δ^{12}-PGJ_2-induced heme oxygenase and p67 syntheses in PAEC. We report here that intracellular GSH suppressed the binding of Δ^{12}-PGJ_2 to nuclei, and inhibited Δ^{12}-PGJ_2-induced heme oxygenase and p67 syntheses in PAEC.

MATERIALS AND METHODS

Materials. L-[^{35}S]Methionine (649 Ci/mmol) and [5,6,8,9,12,14,15-^{3}H]PGD_2 (140 Ci/mmol) were obtained from Du Pont-New England Nuclear (Boston, MA, U.S.A.). L-Buthionine-*S*,*R*-sulfoximine (BSO) was purchased from Sigma (St. Louis, MO, U.S.A.). Unlabeled Δ^{12}-PGJ_2 was from the Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). All other chemicals were of reagent grade. [^{3}H]Δ^{12}-PGJ_2 was generated by incubating [^{3}H]PGD_2 in phosphate-buffered saline with bovine serum albumin

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† Abbreviations: Δ^{12}-PGJ_2, 9-deoxy-$\Delta^{9,12}$-13,14-dihydro-prostaglandin D_2; PAEC, porcine aortic endothelial cells; GSH, glutathione; BSO, buthionine sulfoximine; and SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

(8 mg/mL) overnight at 37°, as described previously [14].

Cell culture. PAEC were prepared from a fresh porcine aorta as described by Gospodarowicz *et al.* [15]. The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, glutamine (4 mM), streptomycin (0.2 mg/mL) and penicillin (100 U/mL) under humidified air containing 5% CO_2 at 37°. Generally, cells at passages 5 to 8 were used for the experiments.

Labeling conditions and gel electrophoresis. Cells cultured in 35-mm dishes (5×10^5 cells/dish) were exposed to the test agents for 3 hr. Thereafter, medium was replaced with 1 mL of methionine-free Eagle's medium containing [^{35}S]methionine (10 μCi/mL) and 10% dialyzed fetal bovine serum, followed by incubation for 2 hr at 37°. After incubation, the cells were washed twice with ice-cold phosphate-buffered saline, scraped from each dish with a rubber policeman and then centrifuged. The cell pellet was lysed and subjected to SDS–10%PAGE, as described previously [16]. The amounts of p31 and p67 synthesized were determined by scanning the fluorograms with a densitometer (Shimadzu CS-900), as described previously [8].

GSH content assay. The intracellular GSH content was determined according to the method of Griffith [17] using reduced GSH as a standard. Briefly, total GSH in cells cultured in a 35-mm dish (5×10^5 cells/dish) was extracted with 1 mL of 5% trichloroacetic acid and 0.01 N HCl. The reaction mixture was comprised of 0.1 M sodium phosphate (pH 7.4), 0.2 mM NADPH, 0.6 mM 5,5'-dithiobis 2-nitrobenzoic acid, 5 mM EDTA and 10 μL of the extract. After the mixture had been preincubated for 1 min at 37°, it was further incubated for 7 min at 37° with 0.5 U of GSH reductase (Oriental Yeast Co., Tokyo, Japan). The reaction was stopped by immersion in ice-water, and then the difference in A_{412} was determined with a spectrophotometer.

Subcellular distribution of [^{3}H]Δ^{12}-PGJ$_2$ in PAEC and binding of [^{3}H]Δ^{12}-PGJ$_2$ to isolated nuclei. For determination of the subcellular distribution of [^{3}H]-Δ^{12}-PGJ$_2$, cells cultured in a 35-mm dish (5×10^5 cells/dish) were incubated for 10 min at 37° with the medium containing 10 μM [^{3}H]Δ^{12}-PGJ$_2$ (200,000 dpm). The incubation was terminated by the addition of 25 μL of 1 N HCl. After the cells had been washed four times with ice-cold phosphate-buffered saline and sonicated in 200 μL of 10 mM ammonium acetate (pH 3.0), containing 0.25 M sucrose and 0.1 mM phenylmethylsulfonyl fluoride, the sonicate was fractionated (800 *g* pellet, 100,000 *g* pellet and 100,000 *g* supernatant) as described previously [8]. The radioactivity associated with each pellet was measured. The 100,000 *g* supernatant was extracted twice with ethyl acetate, and then the radioactivities of the aqueous and organic phases of the supernatant were determined. For determination of the binding of [^{3}H]Δ^{12}-PGJ$_2$ to isolated nuclei, nuclei were isolated from PAEC (1×10^6 cells) as described previously [8]. The majority of DNA content was recovered in the nuclear fraction (800 *g* pellet), but other marker enzyme activity for mitochondria, microsomes or cytosol was not detected or was very weak in this fraction [8]. The standard assay mixture was comprised of 10 μM [^{3}H]-Δ^{12}-PGJ$_2$ (100,000 dpm) and 240 μg of isolated nuclei in 0.1 mL of 10 mM potassium phosphate (pH 7.5), unless otherwise stated. After incubation for 10 min at 37°, the mixture was centrifuged at 12,000 *g* for 5 min at 4°, and then washed twice with 10 mM potassium phosphate (pH 7.5); then the radioactivity associated with the pellet was measured.

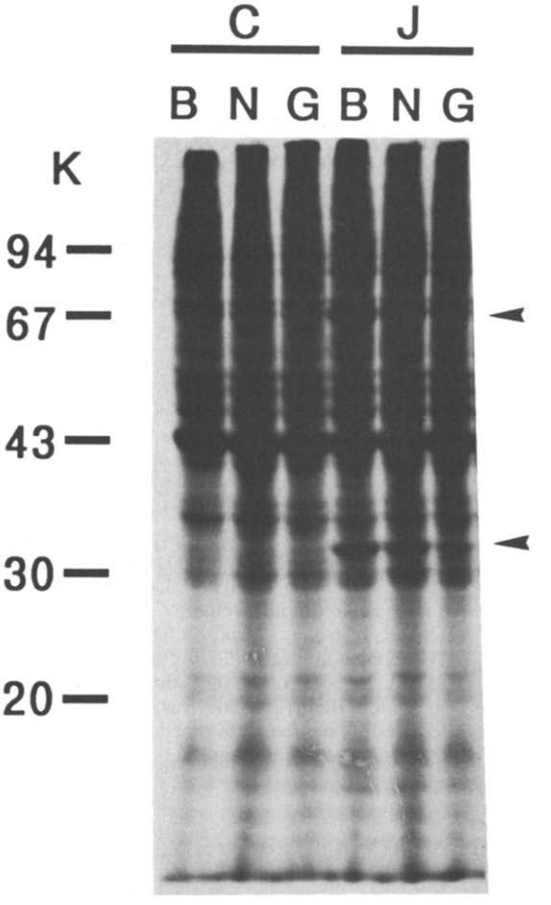

Fig. 1. Effect of BSO or GSH on the induction of p31 and p67 syntheses by Δ^{12}-PGJ$_2$ in PAEC. After PAEC had been preincubated with the vehicle for 12 hr (N), 5 mM BSO for 12 hr (B) or 25 mM GSH for 4 hr (G), they were incubated for 3 hr with (J) or without (C) 10 μM Δ^{12}-PGJ$_2$, and then for a further 2 hr in the presence of [^{35}S]-methionine. The cells were then lysed and subjected to SDS–10%PAGE, followed by fluorography, as described under Materials and Methods. The positions of molecular weight markers are shown on the left. The arrows indicate the positions of p31 and p67.

RESULTS

Effect of intracellular GSH on Δ^{12}-PGJ$_2$-induced protein synthesis in PAEC. We examined the effect of intracellular GSH on Δ^{12}-PGJ$_2$-induced protein synthesis in PAEC. To decrease or increase the level of intracellular GSH, PAEC were exposed to 5 mM BSO, an inhibitor of GSH synthesis, for 12 hr or to 25 mM exogenous GSH for 4 hr, respectively. Figure 1 shows the effect of BSO or GSH treatment of

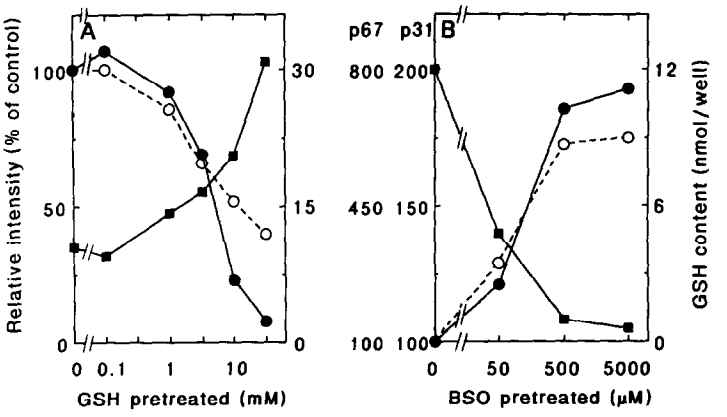

Fig. 2. Concentration dependency of the effects of BSO or GSH on the intracellular GSH content, and the induction of p31 and p67 syntheses by Δ^{12}-PGJ_2 in PAEC. After cells had been preincubated with the indicated concentrations of GSH for 4 hr (A) or of BSO for 12 hr (B), they were incubated for 3 hr with 10 μM Δ^{12}-PGJ_2, and then for a further 2 hr in the presence of [^{35}S]methionine. The cells were then lysed and subjected to SDS–10%PAGE, followed by fluorography, as described under Materials and Methods. The amounts of p31 (○) or p67 (●) synthesized were determined by scanning fluorograms with a densitometer, and are presented as percentages of those in cells without BSO or GSH treatment (A_{460} = 0.45 and 0.32 for p31 and p67, respectively). The cells were incubated with the indicated concentrations of GSH for 4 hr (A) or of BSO for 12 hr (B), and then assayed for the GSH content (■) as described under Materials and Methods. The results shown are representative of three independent experiments.

PAEC on protein synthesis. Δ^{12}-PGJ_2 preferentially induced heme oxygenase (p31) and p67 syntheses. Whereas exposure to BSO or GSH did not alter the basal protein synthesis, BSO markedly enhanced the syntheses of heme oxygenase and p67 induced by Δ^{12}-PGJ_2, but GSH markedly reduced them, suggesting that intracellular GSH modulates the induction of heme oxygenase and p67 syntheses by Δ^{12}-PGJ_2. Thus, we compared the level of intracellular GSH with that of the induction of heme oxygenase and p67 syntheses by Δ^{12}-PGJ_2 in BSO- or GSH-treated cells. As shown in Fig. 2A, GSH treatment progressively increased the level of intracellular GSH up to a 30 mM extracellular GSH, but conversely suppressed the induction of heme oxygenase and p67 syntheses by Δ^{12}-PGJ_2. On the other hand, BSO treatment concentration-dependently decreased the level of intracellular GSH, but conversely potentiated the induction of the syntheses of these proteins. The induction of heme oxygenase and p67 syntheses by Δ^{12}-PGJ_2 appears to be in reciprocal proportion to the level of intracellular GSH. Furthermore, we examined the effect of BSO or GSH treatment on the concentration-dependent curves for Δ^{12}-PGJ_2-induced syntheses of heme oxygenase and p67. As shown in Fig. 3, the half-maximal concentration of Δ^{12}-PGJ_2 for the induction of the syntheses of heme oxygenase and p67 shifted toward the left upon BSO treatment, but in contrast shifted toward the right upon GSH treatment, suggesting that intracellular GSH inhibits Δ^{12}-PGJ_2-induced heme oxygenase and p67 syntheses by reducing the effective concentration of Δ^{12}-PGJ_2.

Effect of intracellular GSH on the accumulation of Δ^{12}-PGJ_2 into nuclei in PAEC. Previously, it was

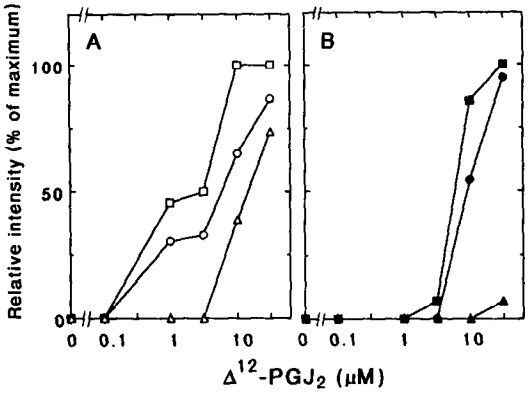

Fig. 3. Effect of GSH or BSO on the concentration dependency of Δ^{12}-PGJ_2 as to the induction of p31 and p67 syntheses in PAEC. After cells had been preincubated with the vehicle for 12 hr (●, ○), 5 mM BSO for 12 hr (■, □) or 25 mM GSH for 4 hr (▲, △), they were incubated for 3 hr with the indicated concentrations of Δ^{12}-PGJ_2, and then for a further 2 hr in the presence of [^{35}S]methionine. The cells were then lysed and subjected to SDS–10%PAGE, followed by fluorography, as described under Materials and Methods. The amounts of p31 (A) or p67 (B) synthesized were determined by scanning fluorograms with a densitometer. The values shown are percentages of the maximal induction (A_{460} = 0.43 and 0.35 for p31 and p67, respectively), and representative of three independent experiments.

reported that Δ^{12}-PGJ_2 is actively transported into cells and accumulates in the nuclei, and that this accumulation is well correlated with the expression of the actions of Δ^{12}-PGJ_2, such as growth inhibition

Table 1. Effect of BSO or GSH on the subcellular distribution of Δ^{12}-PGJ_2 in PAEC

Treatment	GSH (nmool/5 × 10^5 cells)	[^{3}H]Δ^{12}-PGJ_2 (pmol/5 × 10^5 cells) Total	800 *g* ppt	100,000 *g* ppt	100,00 *g* sup Unextractable	100,00 *g* sup Extractable
BSO	1.50 ± 0.20	43.6 ± 4.9	12.3 ± 0.20	6.20 ± 2.1	19.0 ± 2.2	6.05 ± 1.0
GSH	22.5 ± 3.2	56.6 ± 4.1*	8.06 ± 0.20†	8.45 ± 0.30	34.5 ± 3.2†	5.70 ± 1.3

After cells had been preincubated with 5 mM BSO for 12 hr or 25 mM GSH for 4 hr, they were incubated for 10 min at 37° with [^{3}H]Δ^{12}-PGJ_2. The subcellular distribution of Δ^{12}-PGJ_2 accumulated in the cells was determined as described under Materials and Methods. The values shown are means ± SEM for triplicate experiments. Student's *t*-test was used for determining [^{3}H]Δ^{12}-PGJ_2 distribution.

*† Significant differences between BSO and GSH treatment are represented as follows: (*) $P < 0.05$; and (†) $P < 0.01$.

[4–6]. Therefore, we next examined the effect of intracellular GSH on the uptake of Δ^{12}-PGJ_2 into PAEC and its accumulation in nuclei. Table 1 shows the subcellular distribution of accumulated [^{3}H]Δ^{12}-PGJ_2 after [^{3}H]Δ^{12}-PGJ_2 had been transported into BSO- or GSH-treated cells. Whereas the radioactivities of [^{3}H]Δ^{12}-PGJ_2 in the total and 100,000 *g* supernatant fractions of GSH-treated cells (high level of intracellular GSH) were higher than those of BSO-treated cells (low level of intracellular GSH), the amount of [^{3}H]Δ^{12}-PGJ_2 accumulated in the nuclear fraction (800 *g* pellet) of GSH-treated cells was significantly lower than that of BSO-treated cells. The majority of [^{3}H]Δ^{12}-PGJ_2 recovered in the 100,000 *g* supernatant fraction of both BSO- and GSH-treated cells could not be extracted with ethyl acetate at acidic pH, indicating that most Δ^{12}-PGJ_2 is conjugated with GSH or thiol compounds in the cytosol. As judged from these results, intracellular GSH enhanced the total uptake of Δ^{12}-PGJ_2, but intracellular GSH formed a conjugate with Δ^{12}-PGJ_2 transported into cells and suppressed its accumulation in nuclei. Furthermore, treatment of Δ^{12}-PGJ_2 with GSH concentration dependently inhibited the Δ^{12}-PGJ_2 binding to isolated nuclei in reciprocal proportion to the formation of GSH conjugate (data not shown). We next examined the effect of *N*-ethylmaleimide (NEM), a thiol-alkylating agent, on the binding of Δ^{12}-PGJ_2 to isolated nuclei. As shown in Fig. 4, treatment of isolated nuclei with NEM concentration dependently inhibited the binding of Δ^{12}-PGJ_2 to the nuclei, the maximal inhibition being 60% at 5 mM NEM.

Effect of intracellular GSH on the induction of heme oxygenase and p67 syntheses by arsenite and diethylmaleate. We recently reported that the thiol-reactive agents, arsenite and diethylmaleate also induce the syntheses of heme oxygenase and p67 in PAEC [8]. Thus, we examined the effect of intracellular GSH on these thiol-reactive agent-induced heme oxygenase and p67 syntheses. As shown in Fig. 5, BSO treatment markedly potentiated the arsenite- or diethylmaleate-induced syntheses, and GSH treatment suppressed them. We also reported that hemin induces the synthesis of heme oxygenase [9], but GSH treatment very faintly suppressed it (data not shown).

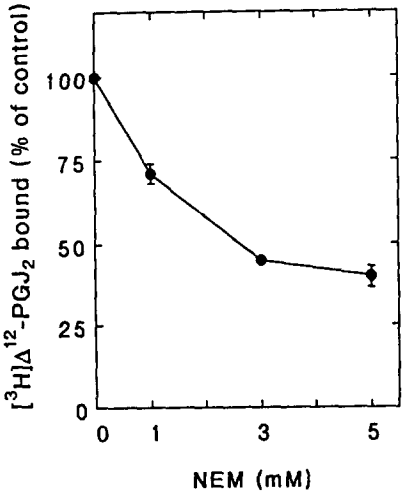

Fig. 4. Effect of preincubation of isolated nuclei with NEM on [^{3}H]Δ^{12}-PGJ_2 binding to the nuclei. After isolated nuclei (240 μg) had been preincubated with the indicated concentrations of NEM for 30 min at 37°, they were incubated with 10 μM [^{3}H]Δ^{12}-PGJ_2 for 10 min at 37°. The binding of [^{3}H]Δ^{12}-PGJ_2 to the nuclei was assayed as described under Materials and Methods. Values are means ± SE for triplicate experiments. Control value: 240 ± 3.0 pmol/240 μg.

DISCUSSION

It has been demonstrated in our laboratory that Δ^{12}-PGJ_2 preferentially induces the synthesis of a 31-kDa heme oxygenase [9], as well as a 67-kDa heat shock protein in PAEC [8]. In the present study, we demonstrated that intracellular GSH suppresses the Δ^{12}-PGJ_2-induced syntheses of these proteins by blocking the binding of Δ^{12}-PGJ_2 to nuclei.

The increase and decrease in the level of intracellular GSH as a consequence of exogenous GSH and BSO treatment, respectively, were well correlated with the suppression and potentiation of

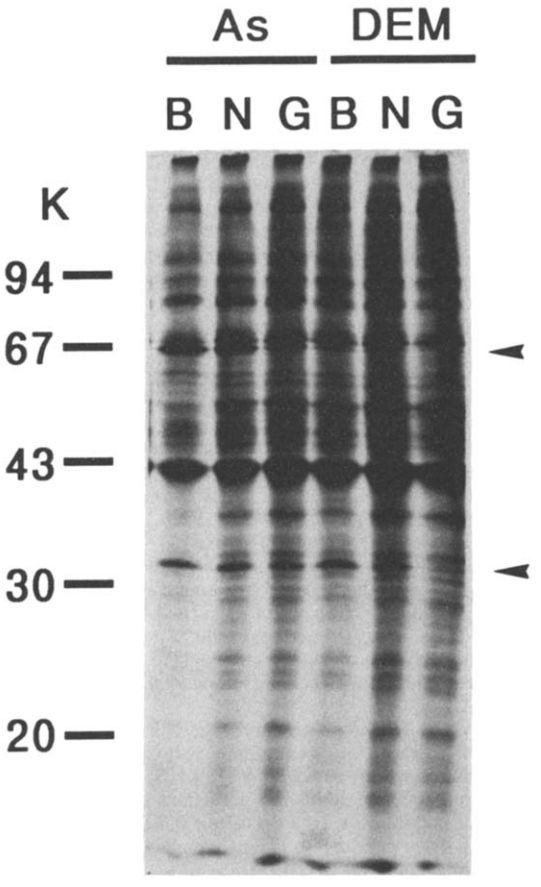

Fig. 5. Effect of BSO or GSH on the induction of p31 and p67 syntheses by arsenite or diethylmaleate in PAEC. After cells had been preincubated with the vehicle for 12 hr (N), 5 mM BSO for 12 hr (B) or 25 mM GSH for 4 hr (G), they were incubated for 3 hr with 10 μM arsenite (As) or 100 μM diethylmaleate (DEM), and then for a further 2 hr in the presence of [^{35}S]methionine. The cells were then lysed and subjected to SDS–10%PAGE, followed by fluorography, as described under Materials and Methods. The positions of molecular weight markers are shown on the left. The arrows indicate the positions of p31 and p67.

Δ^{12}-PGJ_2-induced heme oxygenase and p67 syntheses in PAEC (Fig. 2), suggesting that intracellular GSH negatively regulates the Δ^{12}-PGJ_2-induced protein syntheses. It has been reported that Δ^{12}-PGJ_2 is largely conjugated with intracellular GSH in Chinese hamster ovary cells and hepatoma tissue culture cells, and that depletion of intracellular GSH in these cells upon treatment with BSO and diethylmaleate enhances their sensitivity to the antiproliferative effect of Δ^{12}-PGJ_2 [13], suggesting that GSH suppresses the growth inhibitory effect of Δ^{12}-PGJ_2 by reducing the effective concentration of Δ^{12}-PGJ_2 by forming a conjugate with Δ^{12}-PGJ_2. In PAEC, Δ^{12}-PGJ_2 was also a strong growth inhibitor, and its half-maximal concentration for the inhibition was comparable with that for the induction of p67 (data not shown). Considering that the antiproliferative activity of Δ^{12}-PGJ_2 is due to the induction of 70-kDa heat shock proteins [7], our finding of potentiation by BSO of Δ^{12}-PGJ_2-induced 67-kDa heat shock protein synthesis (Figs. 1 and 2) suggests that BSO treatment decreases the level of intracellular GSH and potentiates the antiproliferative activity of Δ^{12}-PGJ_2 by promoting Δ^{12}-PGJ_2-induced heat shock protein synthesis.

On the other hand, it has been shown that Δ^{12}-PGJ_2 is accumulated in L-1210 murine leukemia cells, most of which is recovered in the nuclei in an ethyl acetate-unextractable form, and that this accumulation of Δ^{12}-PGJ_2 in the nuclei is closely associated with growth inhibition of L-1210 cells by Δ^{12}-PGJ_2 [6]. Therefore, the site of action of intracellular GSH is assumed to be accumulation of Δ^{12}-PGJ_2 in nuclei. As shown in Table 1, the increase in the level of intracellular GSH significantly suppressed the accumulation of Δ^{12}-PGJ_2 in the nuclear fraction of PAEC. In contrast, the intracellular GSH increased the total uptake of Δ^{12}-PGJ_2 into the cells, which is ascribable to the increase in the ethyl acetate-unextractable form of Δ^{12}-PGJ_2 in the cytosol, probably the GSH-conjugated form of Δ^{12}-PGJ_2. Therefore, GSH appears to promote the uptake of Δ^{12}-PGJ_2, and the majority of the transported Δ^{12}-PGJ_2 is in the GSH-conjugated form. In Chinese hamster ovary cells, a thiol-reactive agent, diethylmaleate, has been shown to induce the syntheses of 32 kDa stress proteins and to require the formation of a GSH conjugate for this induction [18], and it was reported that GSH participates in the induction of the syntheses of a 30- to 35-kDa stress protein by chemical inducers in fibroblasts [19], suggesting that the GSH conjugate with the inducers is responsible for the protein induction. In PAEC, GSH treatment increased the ethyl acetate-unextractable GSH conjugate with Δ^{12}-PGJ_2 (Table 1), but inhibited the accumulation of Δ^{12}-PGJ_2 in nuclei and suppressed Δ^{12}-PGJ_2-induced protein synthesis, demonstrating that the GSH-conjugated form of Δ^{12}-PGJ_2 has no ability to induce the protein synthesis and that free Δ^{12}-PGJ_2 is a possibly active form. Furthermore, the decrease in the level of intracellular GSH upon BSO treatment was not by itself inductive (Fig. 1), and an appreciable reduction in the level of intracellular GSH was not detected in cells exposed to Δ^{12}-PGJ_2 (data not shown), indicating that the Δ^{12}-PGJ_2-induced protein synthesis is not due to a reduction in the level of intracellular GSH.

Furthermore, we examined the effect of GSH on the *in vitro* binding of Δ^{12}-PGJ_2 to isolated nuclei. Treatment of Δ^{12}-PGJ_2 with GSH inhibited the binding of Δ^{12}-PGJ_2 to the nuclei, indicating that the GSH conjugate of Δ^{12}-PGJ_2 cannot bind to nuclei, and that Δ^{12}-PGJ_2 may possibly bind to nuclei as a free form. Therefore, intracellular GSH potently inhibits the Δ^{12}-PGJ_2 binding to nuclei itself and this inhibition possibly causes the suppression of Δ^{12}-PGJ_2-induced protein synthesis. Within L-1210 cells, Δ^{12}-PGJ_2 was transferred to and accumulated in the nuclei, and most of the nuclear Δ^{12}-PGJ_2 bound covalently to protein(s) of chromatin and nuclear matrix, since Δ^{12}-PGJ_2 in the nuclei was

almost completely released upon digestion with proteases and clearly separated from DNA [6]. The inhibitory effect of GSH on Δ^{12}-PGJ_2 binding to nuclei suggests that the binding moiety of Δ^{12}-PGJ_2 for nuclear proteins is α,β-unsaturated ketones, with which the thiol residue of GSH associates [12], and thus the binding site of Δ^{12}-PGJ_2 in nuclei comprises the thiol groups of nuclear proteins. Considering that the main binding site of Δ^{12}-PGJ_2 is NEM sensitive, there is a possibility that the binding site of Δ^{12}-PGJ_2 responsible for the protein induction is an NEM-sensitive one, that is, the thiol groups of nuclear proteins.

We recently demonstrated that the thiol-reactive agents arsenite and diethylmaleate also induce heme oxygenase and p67 syntheses in PAEC [8], indicating that a target(s) of the agents is thiol groups in the nuclei. Diethylmaleate forms a thioester conjugate with glutathione [20]. Arsenite is also a thiol binding molecule and can possibly bind to a sulfhydryl group of GSH to form a metal–GSH complex. Intracellular GSH also suppressed arsenite- and diethylmaleate-induced heme oxygenase and p67 syntheses (Fig. 5), suggesting that GSH blocks the binding of these thiol-reactive agents to nuclei, leading to the suppression of protein synthesis. Furthermore, arsenite strongly inhibited the binding of Δ^{12}-PGJ_2 to nuclei (data not shown). Thus, arsenite and Δ^{12}-PGJ_2 may bind to the same thiol groups in the nuclei, and thereby induce the heme oxygenase and p67 syntheses.

Vascular endothelial cells are actively involved in the inflammatory response. On the other hand, mast cells synthesize and subsequently release PGD_2, which is converted into Δ^{12}-PGJ_2 by serum albumin during the process of inflammation. Δ^{12}-PGJ_2 may play an important role as a pathophysiological regulator in the fate of heme liberated during inflammation by the induction of heme oxygenase, and it may inhibit endothelial cell growth by the induction of p67. GSH may regulate the function of Δ^{12}-PGJ_2 in endothelial cells.

Acknowledgements—This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (03671048), and by grants from the Naito Foundation and the CIBA–Geigy Foundation for the Promotion of Science.

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