Biochemical Pharmacology, Vol. 45, No. 12, pp. 2457–2464, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

ACTIVATION OF HEAT SHOCK TRANSCRIPTION FACTORS BY Δ^{12}-PROSTAGLANDIN J_2 AND ITS INHIBITION BY INTRACELLULAR GLUTATHIONE

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(*Received* 20 *November* 1992; *accepted* 23 *February* 1993)

Abstract—We recently showed that Δ^{12}-prostaglandin (PG) J_2 bound to the thiol groups of nuclear proteins and stimulated the synthesis of a 67-kDa heat shock protein (HSP) in porcine aortic endothelial cells, and that intracellular glutathione (GSH) blocked this binding and HSP induction (Koizumi *et al.*, *Biochem Pharmacol* **44**: 1597–1602, 1992). In the present study, we examined the molecular mechanism underlying the induction of HSP by Δ^{12}-PGJ_2. Treatment of cells with Δ^{12}-PGJ_2 induced the activation of heat shock transcription factors (HSF) in a time- and concentration-dependent manner. Cycloheximide pretreatment inhibited this activation. Treatment of cells with buthionine sulfoximine, an inhibitor of GSH synthesis, depleted the intracellular GSH and enhanced the activation of HSF by Δ^{12}-PGJ_2, but treatment with GSH increased the intracellular GSH level and thus reduced the activation. Moreover, the thiol-reactive agents arsenite and diethylmaleate also induced the activation of HSF, and this activation was inhibited by GSH treatment and enhanced by buthionine sulfoximine treatment. These results taken together suggest that Δ^{12}-PGJ_2 binds to the thiol groups of nuclear proteins and activates HSF, leading to the synthesis of the 67-kDa HSP.

9-Deoxy-Δ^9,Δ^{12}-13,14-dihydro-prostaglandin D_2 (Δ^{12}-PGJ_2†), one of the cyclopentenone prostaglandins (PGs) and the ultimate metabolite of prostaglandin D_2 (PGD_2), is a potent inducer of growth inhibition and differentiation of a variety of tumor cells [1, 2]. It is now known that Δ^{12}-PGJ_2 is actively transported into cells and accumulates in the nuclei, where it binds to nuclear proteins, and that this uptake and accumulation are closely correlated with its growth inhibitory effect [3–5]. The growth inhibitory effect of Δ^{12}-PGJ_2 on tumor cells involves the induction of the syntheses of members of the 70-kDa heat shock protein (HSP70) family [6, 7], which causes a block in the progression of the cell cycle from the G_1 to the S phase [8]. Since Δ^{12}-PGJ_2 is formed naturally in the human body [9], it has been proposed that Δ^{12}-PGJ_2 physiologically regulates normal cell growth. We recently found that Δ^{12}-PGJ_2 preferentially induces the synthesis of a 67-kDa HSP and suppresses the proliferation of porcine aortic endothelial cells (PAEC) [10, 11].

A characteristic of Δ^{12}-PGJ_2 is that it contains α,β-unsaturated ketones, and α,β-unsaturated carbonyls are very susceptible to nucleophilic addition reactions with thiols [12, 13]. Recently, we revealed that Δ^{12}-PGJ_2 actively accumulates in the nuclei and binds to the thiol groups of nuclear proteins in PAEC, and that this binding of Δ^{12}-PGJ_2 participates in the synthesis of the 67-kDa HSP [14]. Furthermore, we showed that the most abundant intracellular nonprotein thiol, glutathione (GSH), suppresses Δ^{12}-PGJ_2-induced 67-kDa HSP synthesis by inhibiting the binding of Δ^{12}-PGJ_2 to nuclei in PAEC [14], supporting the importance of Δ^{12}-PGJ_2 binding to nuclear thiol groups in the synthesis of the 67-kDa HSP. However, the molecular mechanism underlying the HSP induction by Δ^{12}-PGJ_2 after binding to the thiol groups remained unknown. On the other hand, induction of HSP by heat shock involves the activation of a heat shock transcription factor (HSF), which binds to a specific region of DNA called the heat shock element (HSE), located upstream of the HSP gene promoter, and induces the transcription of the HSP [15]. Very recently, it was reported that other antiproliferative PGs, PGA_1 and PGA_2, activated HSF in two tumor cell lines, human K562 erythroleukemia cells [16] and growing HeLa S3 cells [17], respectively. In the present work, we show that Δ^{12}-PGJ_2 activated HSF, but in contrast to the case of heat shock, this activation was GSH sensitive and required *de novo* protein synthesis.

MATERIALS AND METHODS

Materials. [γ-^{32}P]ATP (3000 Ci/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA, U.S.A.). Δ^{12}-PGJ_2 was a gift from the Teijin Co. (Tokyo, Japan). Δ^7-PGA_1 was supplied by Dr. M. Suzuki of Nagoya University. Other PGs were purchased from Funakoshi Pharmaceuticals (Tokyo). L-Buthionine-*S*,*R*-sulfoximine (BSO) and GSH were from the Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Nacalai Tesque (Kyoto, Japan), respectively. All other chemicals were of reagent grade.

Cell culture. PAEC were obtained from a fresh

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† Abbreviations: Δ^{12}-PGJ_2, 9-deoxy-Δ^9,Δ^{12}-13,14-dihydro-prostaglandin D_2; PAEC, porcine aortic endothelial cells; GSH, glutathione; HSP, heat shock protein; HSF, heat shock transcription factor; HSE, heat shock element; and BSO, buthionine sulfoximine.

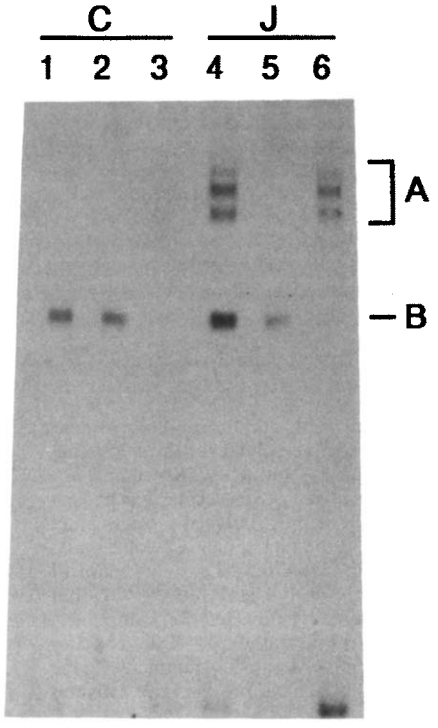

Fig. 1. Effect of Δ^{12}-PGJ_2 on the binding of proteins to HSE in PAEC. PAEC were treated with (J) or without (C) 20 μM Δ^{12}-PGJ_2 for 3 hr. Whole cell lysates (1 and 4), cytosol (2 and 5) and nuclear extracts (3 and 6) were prepared for the gel mobility shift assay using the HSE sequence, as described in Materials and Methods. A and B represent protein–HSE complexes in the nuclear extract and cytosol, respectively.

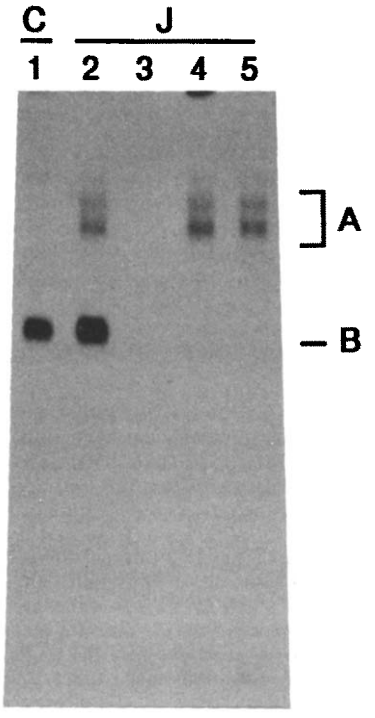

Fig. 2. Effect of single- or double-stranded HSE on Δ^{12}-PGJ_2-induced formation of protein–HSE complexes. Cells were treated with (J) or without (C) 20 μM Δ^{12}-PGJ_2 for 3 hr, and whole cell lysates were prepared. The gel mobility shift assay, using the HSE sequence, was performed in the absence (1 and 2) or presence of a 200-fold excess of unlabeled double-stranded HSE (3), sense single-stranded HSE (4), and antisense single-stranded HSE (5), as described in Materials and Methods. A and B represent protein–HSE complexes in the nuclear extract and cytosol, respectively.

porcine aorta as described by Gospodarowicz *et al.* [18]. 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) in humidified air containing 5% CO_2 at 37°. Generally, cells at passages 4–8 were used for the experiments.

Preparation of nuclear extracts and whole cell lysates. Nuclear extracts were prepared from PAEC cultured in 35-mm dishes (5×10^5 cells/dish) according to Schreiber *et al.* [19]. The cells were washed twice with ice-cold phosphate-buffered saline, scraped from the dishes with a rubber policeman, and then centrifuged. The cell pellet was washed with 10 mM Tris, pH 7.5, containing 130 mM NaCl, 5 mM KCl and 8 mM $MgCl_2$, and resuspended in 160 μL of 20 mM Hepes, pH 7.9, containing 5 mM KCl, 0.5 mM $MgCl_2$ and 0.5 mM phenylmethylsulfonyl fluoride by gentle pipetting. After a 15-min incubation at 0°, 10 μL of a 10% (w/v) solution of NP-40 was added, followed by vigorous vortexing for 10 sec. The mixture was centrifuged at 10,000 *g* for 5 min, and the supernatant was used as the cytosol. The pellet was resuspended in 40 μL of 20 mM Hepes, pH 7.9, containing 25% (w/v) glycerol, 0.5 M NaCl, 1.5 mM $MgCl_2$, 1.5 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/mL leupeptin, 1 μg/mL aprotinin and 1 μM benzamidine, followed by shaking for 30 min at 0°, and then centrifugation at 12,000 *g* for 10 min. The supernatant was used as the nuclear extract. Whole cell lysates were prepared according to the method of Blake *et al.* [20]. Cells cultured in 35-mm dishes were suspended in 50 μL of the extraction buffer for the nuclear extract, sonicated, and then centrifuged at 100,000 *g* for 15 min. The supernatant was used as the whole cell lysate.

Gel mobility shift assay. A complementary pair of DNA fragments containing the sequences, 5′-GATCCTCTGGAACCTTCCAGAG-3′ and 5′-GATCCTCTGGAAGGTTCCAGAG-3′ (corresponding to the consensus HSE sequence [21]), was synthesized as a probe for the gel mobility shift assay using an ABI 391 DNA synthesizer (Applied Biosystems, Inc., CA, U.S.A.), annealed, and then radiolabeled at the 5′ end with [γ-^{32}P]ATP using T_4 polynucleotide kinase. The nuclear extract, whole cell lysate or cytosol (5 μg of protein) was incubated with 2 μg of poly(dI-dC) and 1 ng of the ^{32}P-labeled

probe (10,000 cpm) for 45 min at 30° in 20 μL of 25 mM Hepes, pH 7.9, containing 0.5 mM EDTA, 50 mM KCl, 10% glycerol, 0.5 mM dithiothreitol and 0.5 mM phenylmethylsulfonyl fluoride. For the competition assay, a 200-fold molar excess of the unlabeled probe was incubated with the sample prior to addition of the labeled probe. Reaction mixtures were electrophoresed on native 4% polyacrylamide gels at 4° at 150 V for 2 hr in 50 mM Tris, pH 8.5, containing 380 mM glycine and 2 mM EDTA. The gels were dried on Whatman 3MM paper and autoradiographed with X-ray film (Fuji RX). The amounts of radioactive complexes were determined with a Fuji BAS 2000 imaging analyzer (Fuji Film Co., Tokyo).

Miscellaneous. Intracellular GSH contents were determined according to the method of Griffith [22] as described previously [14]. Protein concentrations were determined according to Lowry *et al.* [23] with bovine serum albumin as the standard.

RESULTS

Effect of Δ^{12}-PGJ_2 on the activation of HSF. To determine whether Δ^{12}-PGJ_2 activates HSF in PAEC, we performed a gel mobility shift assay using a synthetic oligonucleotide probe including the consensus HSE sequence. As shown in Fig. 1, Δ^{12}-PGJ_2 markedly induced three protein–HSE complexes that were derived from the nuclear extract (A in Fig. 1). Heat shock (43°, 60 min) induced the same protein–HSE complexes in the nuclear extract (data not shown). In contrast, constitutive HSE-binding activity was found in the cytosol (B in Fig. 1). We further examined the binding specificity of these HSE binding proteins for single- or double-stranded HSE. The Δ^{12}-PGJ_2-induced formation of ^{32}P-labeled complex A was inhibited by an excess of unlabeled double-stranded HSE, but not by unlabeled single-stranded HSE (both sense and antisense strands) (Fig. 2). Furthermore, the TPA-response element could not inhibit the Δ^{12}-PGJ_2-induced formation of complex A (data not shown). These results indicate that Δ^{12}-PGJ_2 markedly activates three HSF that specifically bind to double-stranded HSE. On the other hand, constitutive protein–HSE complex B in the cytosol was displaced by both single- and double-stranded HSE. We then examined the time courses and concentration dependencies of Δ^{12}-PGJ_2-induced formation of the HSF–HSE complexes. As shown in Fig. 3, Δ^{12}-PGJ_2 induced the formation of three HSF–HSE complexes with similar time courses, the levels being more than 20-fold over the respective basal levels by 6 hr. Δ^{12}-PGJ_2 concentration-dependently induced the formation of these complexes, and they showed similar concentration–response curves (Fig. 4). These concentration dependencies were consistent with that of 67-kDa HSP induction by Δ^{12}-PGJ_2 [11].

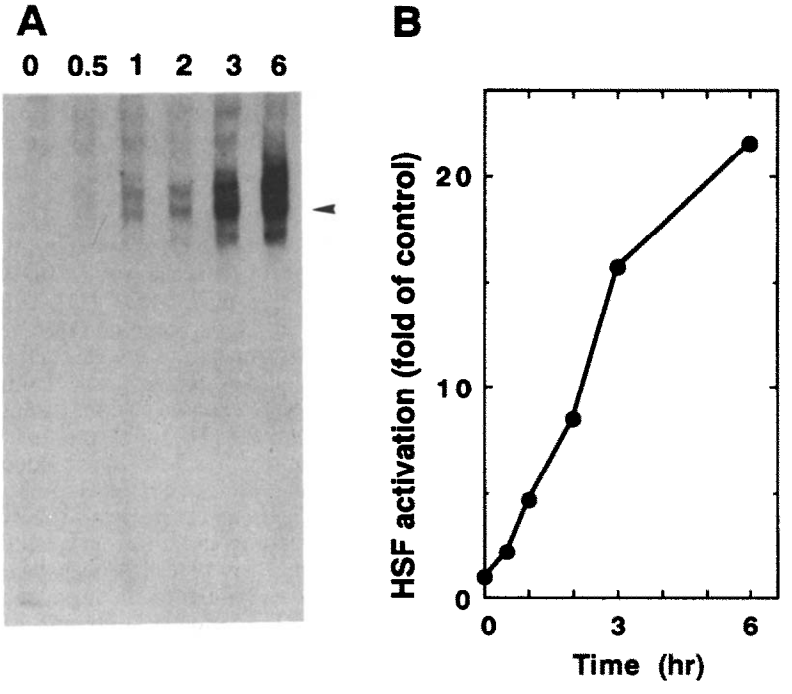

Fig. 3. Time course of the Δ^{12}-PGJ_2-induced activation of HSF. (A) Cells were treated with 20 μM Δ^{12}-PGJ_2 for the indicated times (hr). Nuclear extracts were prepared for the gel mobility shift assay using the HSE sequence, as described in Materials and Methods. (B) The amounts of three HSF–HSE complexes with different mobilities were determined with a Fuji BAS 2000 imaging analyzer, but only the results for the complex with the intermediate mobility (arrow) are shown. Values are expressed as fold of control (0 hr), and are representative of three independent experiments. The other two complexes exhibited similar time-course curves.

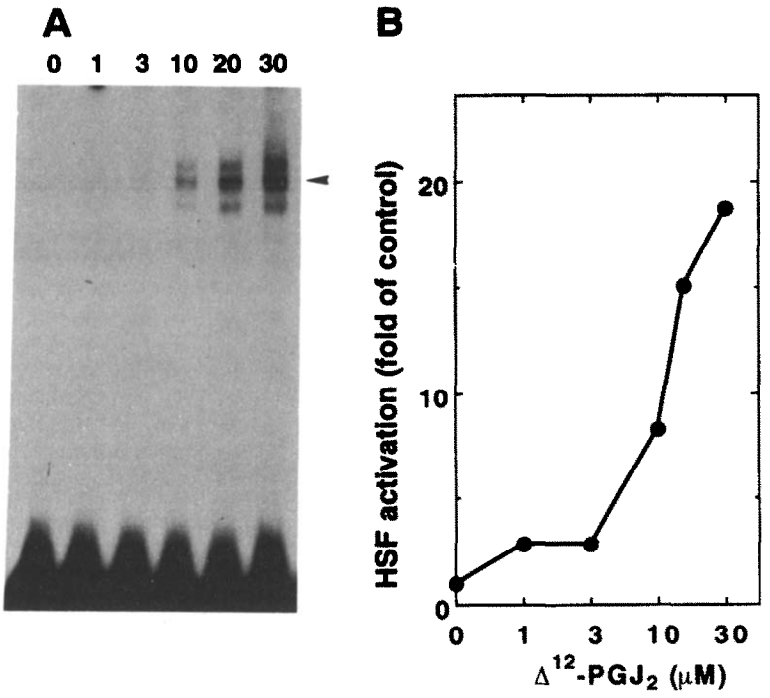

Fig. 4. Concentration dependency of the Δ^{12}-PGJ_2-induced activation of HSF. (A) Cells were treated for 3 hr with the indicated concentrations (μM) of Δ^{12}-PGJ_2. Nuclear extracts were prepared for the gel mobility shift assay using the HSE sequence, as described in Materials and Methods. (B) The amounts of three HSF–HSE complexes with different mobilities were determined with a Fuji BAS 2000 imaging analyzer, but only the results for the complex with the intermediate mobility (arrow) are shown. Values are expressed as fold of control (0 μM), and are representative of three independent experiments. The other two complexes exhibited similar concentration-dependent curves.

We have already shown that Δ^{12}-PGJ_2 was accumulated in the nuclei and bound to nuclear proteins in PAEC [14]. When the nuclei of PAEC were incubated *in vitro* with [^{3}H]Δ^{12}-PGJ_2, several proteins covalently conjugated with [^{3}H]Δ^{12}-PGJ_2 could be found about 30 kDa on a sodium dodecyl sulfate–polyacrylamide gel (data not shown). However, the mechanism(s) for activation of HSF after Δ^{12}-PGJ_2 binding to nuclear proteins is unknown. To determine whether the formation of the HSF–HSE complexes by Δ^{12}-PGJ_2 requires *de novo* protein synthesis, we examined the effect of cycloheximide on the formation. Prior treatment of the cells with cycloheximide almost completely inhibited the Δ^{12}-PGJ_2-induced formation of the HSF–HSE complexes, whereas the heat shock-induced formation of the complexes was not inhibited by cycloheximide treatment (data not shown).

We previously reported that the induction of the 67-kDa HSP was specific for cyclopentenone PGs in PAEC [10]. We then examined the effects of various PGs on the formation of the HSF–HSE complexes. As shown in Fig. 5, the complex formation was induced by PGs in the following order: Δ^7-$PGA_1 \geqq \Delta^{12}$-$PGJ_2 > PGA_2 > PGA_1$. PGD_2, PGE_1 and PGE_2 also induced the complex formation, but the levels were very low. However, $PGF_{2\alpha}$ and carbacyclin, a stable PGI_2 analog, had no induction activity. These results indicate that the induction of the HSF–HSE complexes is specific for cyclopentenone PGs, and Δ^7-PGA_1 and Δ^{12}-PGJ_2 activate HSF more potently than PGA_1 and PGA_2 among the cyclopentenone PGs.

Effect of intracellular GSH on the formation of the HSF–HSE complexes. We recently reported that intracellular GSH suppresses the induction of the 67-kDa HSP by Δ^{12}-PGJ_2 [14]. Thus, we examined the effect of intracellular GSH on the Δ^{12}-PGJ_2-induced formation of the HSF–HSE complexes. To reduce or elevate the level of intracellular GSH, PAEC were exposed to 5 mM BSO, an inhibitor of GSH synthesis [24], for 12 hr or to 25 mM exogenous GSH for 4 hr. BSO treatment induced a decrease in the intracellular GSH level from 10 to 1.0 nmol/5×10^5 cells, and GSH treatment conversely induced an increase in the intracellular GSH level from 10 to 30 nmol/5×10^5 cells. As shown in Fig. 6, whereas BSO or GSH treatment itself did not induce the complex formation, BSO treatment potentiated the Δ^{12}-PGJ_2-induced formation of the HSF–HSE complexes and, in contrast, GSH treatment reduced it. Short-time treatment with BSO (6 hr) itself also did not induce the formation (data not shown). These results suggest that intracellular GSH suppresses the induction of the 67-kDa HSP by Δ^{12}-PGJ_2 through the inhibition of Δ^{12}-PGJ_2-induced formation of HSF–HSE complexes.

We recently reported that the thiol-reactive agents

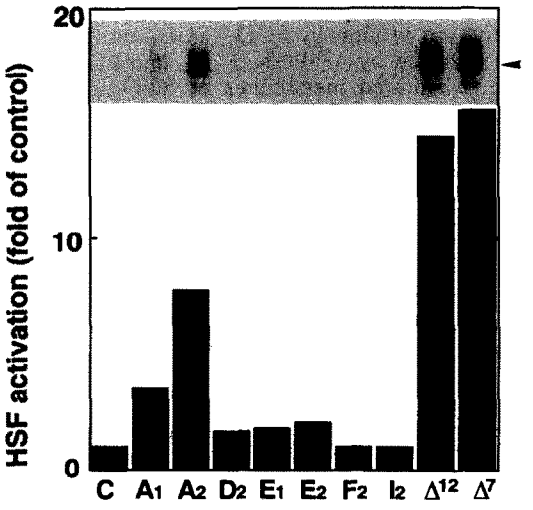

Fig. 5. Effects of various PGs on the activation of HSF. Cells were treated for 3 hr with the vehicle (C), PGA_1 (A_1), PGA_2 (A_2), PGD_2 (D_2), PGE_1 (E_1), PGE_2 (E_2), $PGF_{2\alpha}$ (F_2), carbacyclin (I_2), Δ^{12}-PGJ_2 (Δ^{12}), and Δ^7-PGA_1 (Δ^7) at 20 μM. Nuclear extracts were prepared for the gel mobility shift assay using the HSE sequence, as described in Materials and Methods. The amounts of the HSF–HSE complex with the intermediate mobility (arrow) were determined with a Fuji BAS 2000 imaging analyzer. Values are expressed as fold of control, and are representative of three independent experiments.

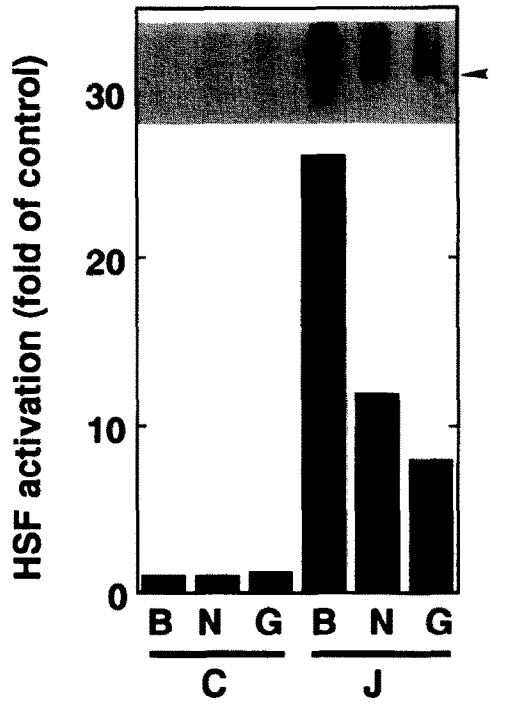

Fig. 6. Effect of BSO or GSH on the Δ^{12}-PGJ_2-induced activation of HSF. Cells were treated with the vehicle for 12 hr (N), 5 mM BSO for 12 hr (B), or 25 mM GSH for 4 hr (G). After BSO or GSH had been removed, they were treated with (J) or without (C) 20 μM Δ^{12}-PGJ_2 for 3 hr. Nuclear extracts were prepared for the gel mobility shift assay using the HSE sequence, as described in Materials and Methods. The amounts of the HSF–HSE complex with the intermediate mobility (arrow) were determined with a Fuji BAS 2000 imaging analyzer. Values are expressed as fold of control, and are representative of three independent experiments.

arsenite and diethylmaleate also induced the 67-kDa HSP in PAEC in an intracellular GSH-sensitive manner [14]. Thus, we examined whether arsenite or diethylmaleate activates HSF, and whether intracellular GSH modulates the action of these compounds in PAEC. As shown in Fig. 7, arsenite and diethylmaleate induced the same HSF–HSE complexes as Δ^{12}-PGJ_2 did, and BSO treatment enhanced the formation of the HSF–HSE complexes by these compounds, but GSH treatment suppressed it. The level of intracellular GSH was reduced by 20 and 65% in the cells exposed to arsenite and diethylmaleate, respectively. In contrast, arsenite activated HSF more potently than diethylmaleate (Fig. 7), suggesting that this thiol-reactive agent-induced activation of HSF is not due to a reduction in the level of intracellular GSH. Cycloheximide also inhibited the formation of the HSF–HSE complexes by these compounds (data not shown). These results indicate that thiol-reactive agent-induced activation of HSF is GSH sensitive and requires *de novo* protein synthesis.

DISCUSSION

It has been demonstrated in our laboratory that Δ^{12}-PGJ_2 preferentially induces a 67-kDa HSP in PAEC [10] and intracellular GSH suppresses the Δ^{12}-PGJ_2-induced 67-kDa HSP synthesis by inhibiting the binding of Δ^{12}-PGJ_2 to the nuclei [14]. In the present study, we showed that Δ^{12}-PGJ_2 activates HSF in a *de novo* protein synthesis-dependent manner and that intracellular GSH inhibits the Δ^{12}-PGJ_2-induced activation of HSF in PAEC.

The gel mobility shift assay revealed that Δ^{12}-PGJ_2 markedly induced the activation of three HSF that specifically bind to double-stranded HSE (Fig. 1). Since the nuclear extract of PAEC was prepared in the presence of various protease inhibitors, the three HSF appear not to be products of proteolysis of the highest molecular weight HSF, and thus they must be different proteins. Recently, it was shown that there are at least three HSF-related genes in tomatoes [25] and two HSF genes in humans [26, 27], but these HSF show the same binding specificity for HSE. In PAEC, the difference between the three HSF responsible for the 67-kDa HSP induction remains unknown. It is known that Δ^{12}-PGJ_2 is actively transported into cells and accumulated in the nuclei, where it covalently binds to nuclear proteins [3–5]. We also showed that Δ^{12}-PGJ_2 is accumulated in the nuclei and bound to nuclear proteins in PAEC, and that this nuclear accumulation is closely correlated with the induction of the 67-kDa HSP and the subsequent growth inhibition of

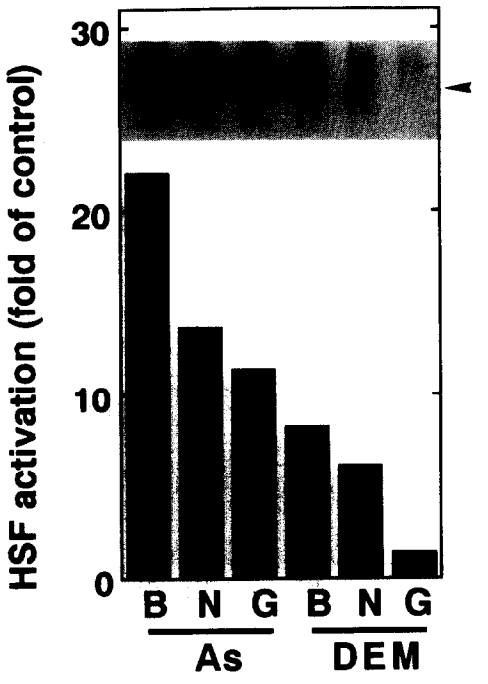

Fig. 7. Effect of BSO or GSH on arsenite- or diethylmaleate-induced activation of HSF. Cells were treated with the vehicle for 12 hr (N), 5 mM BSO for 12 hr (B), or 25 mM GSH for 4 hr (G). After BSO or GSH had been removed, they were treated for 3 hr with 20 μM arsenite (As) or 150 μM diethylmaleate (DEM). Nuclear extracts were prepared for the gel mobility shift assay using the HSE sequence, as described in Materials and Methods. The amounts of the HSF–HSE complex with the intermediate mobility (arrow) were determined with a Fuji BAS 2000 imaging analyzer. Values are expressed as fold of control of Fig. 6, and are representative of three independent experiments.

PAEC [14]. Furthermore, we revealed that Δ^{12}-PGJ_2 covalently binds to several proteins with molecular masses of around 30 kDa in the nuclei of PAEC. The nuclear accumulation of Δ^{12}-PGJ_2 is reminiscent of the action of steroid hormones or dioxins, which bind to their receptors, followed by the binding of the liganded receptors to their specific DNA response elements [28, 29]. *In vitro* treatment of a nuclear extract with dioxin induces the binding of its receptor to the corresponding response element [29]. The Δ^{12}-PGJ_2-induced activation of HSF required *de novo* protein synthesis. Therefore, Δ^{12}-PGJ_2 appears to induce the synthesis of a protein(s) that is responsible for the activation of HSF.

It was reported recently that PGA_1 [16] and PGA_2 [17] activate HSF in two tumor cell lines, human K562 erythroleukemia cells and growing HeLa S3 cells, respectively, the activation of HSF being also dependent on protein synthesis. Only PGA_1 and PGA_2 were effective in activating HSF and inducing HSP70 expression in these cells; PGE and PGD were ineffective. However, the effect of Δ^{12}-PGJ_2 has yet to be determined. Among the various PGs tested, the activation of HSF was specific for cyclopentenone PGs in PAEC (Fig. 5). The weak activation of HSF by PGD_2, PGE_1 and PGE_2 may be mediated by Δ^{12}-PGJ_2, PGA_1 and PGA_2, which are dehydrated metabolites of PGD_2, PGE_1 and PGE_2, respectively [1, 2]. Good correlation was observed between the specificities of PGs for the activation of HSF and for the induction of the 67-kDa HSP [10]. Furthermore, we determined that Δ^{12}-PGJ_2 and Δ^{7}-PGA_1 activated HSF more potently than PGA_1 and PGA_2 among the cyclopentenone PGs. A characteristic of cyclopentenone PGs is that they contain α,β-unsaturated ketones, which are very susceptible to nucleophilic addition reactions with thiols [12, 13]. We recently reported that the binding of Δ^{12}-PGJ_2 to the nuclear proteins is *N*-ethylmaleimide sensitive in PAEC, indicating that the binding site of Δ^{12}-PGJ_2 in the nuclei is a thiol group [14]. PGA_1 or PGA_2 forms the mono conjugate with a thiol [30, 31], but Δ^{12}-PGJ_2 or Δ^{7}-PGA_1 can form the bis conjugate with two thiols [12, 13]. Furthermore, the binding of PGA_1 to synthetic polymer-supported thiols is reversible, but that of Δ^{12}-PGJ_2 or Δ^{7}-PGA_1 is irreversible.* In fact, it has been reported that the association of PGA_2 with nuclei is reversible, but that of Δ^{12}-PGJ_2 is irreversible [5]. The stronger activation of HSF and the resultant induction of the 67-kDa HSP by Δ^{12}-PGJ_2 and Δ^{7}-PGA_1 may be ascribed to their ability to form stable bis conjugates with a thiol group. Intracellular GSH plays an important role in the regulation of the action of anticancer drugs through direct conjugation with them [32]. In our previous study, we demonstrated that intracellular GSH suppresses the Δ^{12}-PGJ_2-induced synthesis of the 67-kDa HSP by blocking the binding of Δ^{12}-PGJ_2 to the thiol groups of nuclear proteins in PAEC [14]. Intracellular GSH also suppressed the Δ^{12}-PGJ_2-induced activation of HSF in PAEC (Fig. 6). Thus, the inhibition by intracellular GSH of the Δ^{12}-PGJ_2-induced 67-kDa HSP synthesis is due to the inhibition of the Δ^{12}-PGJ_2-induced activation of HSF. The decrease in the level of intracellular GSH upon BSO treatment by itself did not activate HSF (Fig. 6). We previously showed that an appreciable reduction in the level of intracellular GSH is not detected in cells exposed to Δ^{12}-PGJ_2 [14]. Therefore, the Δ^{12}-PGJ_2-induced activation of HSF is not due to a secondary effect of a reduction in the level of intracellular GSH. Considering these results, it is assumed that the binding of Δ^{12}-PGJ_2 to the thiol groups of nuclear proteins is the trigger for the activation of HSF, and that the conjugate of Δ^{12}-PGJ_2 with intracellular GSH makes Δ^{12}-PGJ_2 inaccessible to the thiol group.

We recently demonstrated that the thiol-reactive agents arsenite and diethylmaleate also induce 67-kDa HSP synthesis [10], and that intracellular GSH suppresses arsenite- and diethylmaleate-induced 67-kDa HSP synthesis [14]. Diethylmaleate forms a thioester conjugate with GSH [33]. Arsenite is also a thiol-binding molecule and can possibly bind to a sulfhydryl group of GSH to form a metal–GSH complex. Arsenite and diethylmaleate also induced the activation of HSF, and intracellular GSH

* Personal communication from Dr. M. Suzuki of Nagoya University, cited with permission.

suppressed the activation of HSF by these compounds (Fig. 7), suggesting that their binding to the thiol groups of nuclear proteins induces the activation of HSF. Considering the requirement of *de novo* protein synthesis for both Δ^{12}-PGJ$_2$ and thiol-reactive agent-induced activation of HSF, they must bind to the thiol groups of nuclear proteins and their binding may induce the synthesis of a protein(s) that is responsible for the activation of the HSF in PAEC.

Acknowledgements—We would like to thank Dr. M. Suzuki of Nagoya University for supplying the Δ^7-PGA$_1$, and Mr. S. Nakagawa of this laboratory for the technical suggestions regarding the gel mobility shift assay. 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.

REFERENCES

1. Narumiya S and Fukushima M, Δ^{12}-Prostaglandin J$_2$, an ultimate metabolite of prostaglandin D$_2$ exerting cell growth inhibition. *Biochem Biophys Res Commun* **127**: 739–745, 1985.
2. Santoro MG, Benedetto A and Jaffe BM, Prostaglandin A$_1$ induces differentiation in Friend erythroleukemia cells. *Prostaglandins* **17**: 719–727, 1979.
3. Narumiya S and Fukushima M, Site and mechanism of growth inhibition by prostaglandins. I. Active transport and intracellular accumulation of cyclopentenone prostaglandins, a reaction leading to growth inhibition. *J Pharmacol Exp Ther* **239**: 500–505, 1986.
4. Narumiya S, Ohno K, Fukushima M and Fujiwara M, Site and mechanism of growth inhibition by prostaglandins. II. Temperature-dependent transfer of a cyclopentenone prostaglandin to nuclei. *J Pharmacol Exp Ther* **239**: 506–511, 1986.
5. Narumiya S, Ohno K, Fukushima M and Fujiwara M, Site and mechanism of growth inhibition by prostaglandins. III. Distribution and binding of prostaglandin A$_2$ and Δ^{12}-prostaglandin J$_2$ in nuclei. *J Pharmacol Exp Ther* **242**: 306–311, 1987.
6. Ohno K, Fukushima M, Fujiwara M and Narumiya S, Induction of 68,000-dalton heat shock proteins by cyclopentenone prostaglandins. Its association with a prostaglandin-induced G$_1$ block in cell cycle progression. *J Biol Chem* **263**: 19764–19770, 1988.
7. Santoro MG, Garaci E and Amici C, Prostaglandin with antiproliferative activity induces the synthesis of a heat shock protein in human cells. *Proc Natl Acad Sci USA* **86**: 8407–8411, 1989.
8. Ohno K, Sakai T, Fukushima M, Narumiya S and Fujiwara M, Site and mechanism of growth inhibition by prostaglandins. IV. Effect of cyclopentenone prostaglandins on cell cycle progression of G$_1$-enriched HeLa S3 cells. *J Pharmacol Exp Ther* **245**: 294–298, 1988.
9. Hirata Y, Hayashi H, Ito S, Kikawa Y, Ishibashi M, Sudo M, Miyazaki H, Fukushima M, Narumiya S and Hayaishi O, Occurrence of 9-deoxy-Δ^9,Δ^{12}-13,14-dihydroprostaglandin D$_2$ in human urine. *J Biol Chem* **263**: 16619–16625, 1988.
10. Koizumi T, Yamauchi R, Irie A, Negishi M and Ichikawa A, Induction of a 31,000-dalton stress protein by prostaglandins D$_2$ and J$_2$ in porcine aortic endothelial cells. *Biochem Pharmacol* **42**: 777–785, 1991.
11. Koizumi T, Negishi M and Ichikawa A, Induction of heme oxygenase by Δ^{12}-prostaglandin J$_2$ in porcine aortic endothelial cells. *Prostaglandins* **43**: 121–131, 1992.
12. Atsmon J, Sweetman BJ, Baertschi SW, Harris TM and Roberts LJ II, Formation of thiol conjugates of 9-deoxy-Δ^9,Δ^{12}(*E*)-prostaglandin D$_2$ and Δ^{12}(*E*)-prostaglandin D$_2$. *Biochemistry* **29**: 3760–3765, 1990.
13. Atsmon J, Freeman ML, Meredith MJ, Sweetman BJ and Roberts LJ II, Conjugation of 9-deoxy-Δ^9,Δ^{12}(*E*)-prostaglandin D$_2$ with intracellular glutathione and enhancement of its antiproliferative activity by glutathione depletion. *Cancer Res* **50**: 1879–1885, 1990.
14. Koizumi T, Negishi M and Ichikawa A, Inhibitory effect of intracellular glutathione on Δ^{12}-prostaglandin J$_2$-induced protein syntheses in porcine aortic endothelial cells. *Biochem Pharmacol* **44**: 1597–1602, 1992.
15. Ang D, Liberek K, Skowyra D, Zylicz M and Georgopoulos C, Biological role and regulation of the universally conserved heat shock proteins. *J Biol Chem* **266**: 24233–24236, 1991.
16. Amici C, Sistonen L, Santoro MG and Morimoto RI, Antiproliferative prostaglandins activate a heat shock transcription factor. *Proc Natl Acad Sci USA* **89**: 6227–6231, 1992.
17. Holbrook NJ, Carlson SG, Choi AMK and Fargnoli J, Induction of HSP70 gene expression by antiproliferative prostaglandin PGA$_2$: A growth-dependent response mediated by activation of a heat shock transcription factor. *Mol Cell Biol* **12**: 1528–1534, 1992.
18. Gospodarowicz D, Moran J, Braun D and Birdwell C, Clonal growth of bovine vascular endothelial cells: Fibroblast growth factor as a survival agent. *Proc Natl Acad Sci USA* **73**: 4120–4124, 1976.
19. Schreiber E, Matthias P, Müller MM and Schaffner W, Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. *Nucleic Acids Res* **17**: 6419, 1989.
20. Blake MJ, Udelsman R, Feulner GJ, Norton DD and Holbrook NJ, Stress-induced heat shock protein 70 expression in adrenal cortex: An adrenocorticotropic hormone-sensitive, age-dependent response. *Proc Natl Acad Sci* **88**: 9873–9877, 1991.
21. Müller RM, Taguchi H and Shibahara S, Nucleotide sequence and organization of the rat heme oxygenase gene. *J Biol Chem* **262**: 6795–6802, 1987.
22. Griffith OW, Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem* **106**: 207–212, 1980.
23. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
24. Griffith OW and Meister A, Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (*S-n*-butyl homocysteine sulfoximine). *J Biol Chem* **254**: 7558–7560, 1979.
25. Scharf KD, Rose S, Zott W, Schöff F and Nover L, Three tomato genes code for heat stress transcription factors with a region of remarkable homology to the DNA-binding domain of the yeast HSF. *EMBO J* **9**: 4495–4501, 1990.
26. Rabindran SK, Giorgi G, Clos J and Wu C, Molecular cloning and expression of a human heat shock factor, HSF1. *Proc Natl Acad Sci USA* **88**: 6906–6910, 1991.
27. Schuetz TJ, Gallo GJ, Sheldon L, Tempst P and Kingston RE, Isolation of a cDNA for HSF2: Evidence for two heat shock factor genes in humans. *Proc Natl Acad Sci USA* **88**: 6911–6915, 1991.
28. Moudgil VK, Phosphorylation of steroid hormone receptors. *Biochim Biophys Acta* **1055**: 243–258, 1990.
29. Wen L-P, Koeiman N and Whitlock JP Jr, Dioxin-inducible, Ah receptor-dependent transcription *in vitro*. *Proc Natl Acad Sci USA* **87**: 8545–8549, 1990.
30. Cagen LM, Fales HM and Pisano JJ, Formation of glutathione conjugates of prostaglandin A$_1$ in human red blood cells. *J Biol Chem* **251**: 6550–6554, 1976.
31. Parker J and Ankel H, Formation of a prostaglandin

A_2-glutathione conjugate in L1210 mouse leukemia cells. *Biochem Pharmacol* **43**: 1053–1060, 1992.
32. Wolf CR, Lewis AD, Carmichael J, Adams DJ, Allan SG and Ansell DJ, The role of glutathione in determining the response of normal and tumor cells to anticancer drugs. *Biochem Soc Trans* **15**: 728–730, 1987.
33. Ormstad K, Jones DP and Orrenius S, Characteristics of glutathione biosynthesis by freshly isolated rat kidney cells. *J Biol Chem* **255**: 175–181, 1980.