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INDUCTION OF A 31,000-DALTON STRESS PROTEIN BY PROSTAGLANDINS D_2 AND J_2 IN PORCINE AORTIC ENDOTHELIAL CELLS

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Abstract—Prostaglandin (PG) D_2 and PGJ_2 stimulated porcine aortic endothelial cells to synthesize a 31,000-dalton protein (termed p31) in a time- and concentration-dependent manner. The induction of p31 synthesis was specific for PGD_2, PGJ_2 and PGA_1 among the various PGs tested. p31 was also synthesized in response to the thiol-reactive agent diethylmaleate and heavy metal sodium arsenite but not to high temperature treatment, platelet-derived growth factor, and 12-*O*-tetradecanoylphorbol 13-acetate. Using two-dimensional polyacrylamide gel electrophoresis, p31 induced by PGJ_2 had an isoelectric point of 5.4, which overlapped exactly with that induced by arsenite. These results taken together indicate that p31 represents one of the stress proteins whose expression is regulated primarily by thio-active compounds but not by hyperthermia. Furthermore, it was induced by PGD_2 and PGJ_2 in rat capillary endothelial cells, rat skin fibroblasts, and rat hepatocytes. The data obtained from this study suggest that p31 induced by PGD_2 and PGJ_2 may play a role in the metabolic regulation of many mammalian cells.

Prostaglandin (PG†) D_2 is ubiquitously distributed in almost all mammalian tissues and organs, and produces a wide range of biological actions [1, 2]. In culture medium, PGD_2 is dehydrated to 9-deoxy-Δ^9-PGD_2 (PGJ_2) and PGJ_2 formed nonenzymatically is further converted by serum albumin to deoxy-$\Delta^{9,12}$-13,14-dihydro-PGD_2 (Δ^{12}-PGJ_2), the ultimate metabolite of PGD_2 [3]. PGD_2 initially was thought to be a very potent antiproliferative agent in various tumor cell lines [4–6], but it was subsequently shown that PGD_2 *per se* was inactive and that the dehydrates of PGD_2, PGJ_2 and Δ^{12}-PGJ_2 were the active forms [7, 8]. It is now known that Δ^{12}-PGJ_2 is actively transported into cells by a specific carrier and accumulates in the nuclei by a temperature-sensitive process [9]. This uptake and accumulation were correlated closely with growth inhibition caused by these PGs. Thus, the primary target(s) of these PGs may be in the nuclei. In connection with this assumption, the protein synthesis inhibitors, cycloheximide and emetine, were reported to have a protective effect against Δ^{12}-PGJ_2 cytotoxicity [10], suggesting that the antitumor effect of Δ^{12}-PGJ_2 involves some specific protein synthesis. Previously, Santoro *et al.* [11] indicated that certain specific proteins in cells are induced by treatment with PGA_1. Furthermore, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of [^{35}S]methionine-incorporated proteins revealed an increased synthesis of 86-, 70- and 66-kD proteins in Δ^{12}-PGJ_2-treated human neuroblastoma cells under conditions in which Δ^{12}-PGJ_2 exerts cytotoxicity [12]. In a series of experiments using HeLa S3 cells enriched in the G_1 phase by serum starvation, it has been demonstrated that Δ^{12}-PGJ_2 extensively induced 68-kD proteins, which are identified as members of the 70-kD heat shock protein group [13]. Although the molecular basis of the inhibition by PGD_2 and its metabolites has been characterized in tumor cells, there have not yet been any reports concerning their effects on the metabolism of normal tissues. Therefore, we investigated the effects of PGD_2 and PGJ_2 on protein synthesis in normal porcine aortic endothelial cells (PAEC), which are actively proliferating. We report here that PGD_2 and PGJ_2 preferentially induced the synthesis of a 31-kD protein as well as a 67-kD protein in PAEC.

MATERIALS AND METHODS

Materials. L-[^{35}S]Methionine (649 Ci/mmol) was obtained from Du Pont-New England Nuclear. 16,16-Dimethyl PGD_2 and its enantiomer were gifts from Ono Pharmaceuticals, Osaka, Japan. ZK110841, BW245C, and AH6809 were supplied by Dr. S. Ito (Osaka Bioscience Institute, Osaka, Japan). PGs were purchased from Funakoshi Pharmaceuticals (Tokyo). Platelet-derived growth factor (PDGF) was from the Toyobo Co. Ltd. (Tokyo, Japan). 12-*O*-Tetradecanoylphorbol 13-acetate (TPA) was from Sigma. Sodium arsenite and diethylmaleate were from Nacalai Tesque (Kyoto). All other chemicals were of reagent grade.

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† Abbreviations: PG, prostaglandin; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PAEC, porcine aortic endothelial cells; PDGF, platelet-derived growth factor; and TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

Cell culture and fractionation. PAEC were collected from a fresh porcine aorta as described by Gospodarowicz *et al.* [14]. Rat capillary endothelial cells were prepared from epidermal fat pads of Sprague–Dawley rats, 3- to 5-weeks-old, essentially according to the procedure of Björntorp *et al.* [15]. These 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 units/mL). Generally, cells from passages 8 to 12 were used for experiments. Parenchymal hepatocytes were isolated from rat liver by collagenase perfusion, and cultured in William's medium E supplemented with 5% calf serum, 0.1 μM insulin and 0.1 μM dexamethasone for 16–24 hr prior to experimentation [16]. Fibroblasts were isolated from rat dorsal skin essentially according to the method of Hunter [17]. For fractionation, the PAEC harvested (1×10^7 cells) were lysed in 75 μL of 10 mM Tris–HCl, pH 7.5, containing 1 mM EDTA, 0.25 M sucrose and 0.1 mM phenylmethylsulfonyl fluoride using a Sonicator (Shibata, SU-10). After centrifugation at 800 *g* for 10 min, the pellet was washed once by recentrifugation as described above in the same medium and then the combined supernatants were centrifuged at 9500 *g* for 10 min. The resulting supernatant was further centrifuged at 100,000 *g* for 60 min. The pellet of each fraction was resuspended in 100 μL of 10 mM potassium phosphate (pH 7.4). The activities of marker enzymes, succinate dehydrogenase [18], NADPH–cytochrome *c* reductase [19] and lactate dehydrogenase [20], and DNA content [21] in each subcellular fraction were measured as described in the literature.

Labeling conditions and gel electrophoresis. Cells cultured in 6-well plates (5×10^5 cells/well) were exposed to various PGs or other agents for 6 hr unless otherwise indicated. Thereafter, medium was replaced with methionine-free Eagle's medium containing [^{35}S]methionine (10 μCi/mL), respective agents and 10% dialyzed fetal bovine serum, and the cells were further incubated for 3 hr at 37°. Cells were washed with ice-cold phosphate-buffered saline three times, scraped from wells with a rubber policeman, and centrifuged. The cell pellet was lysed in 40 μL of 10 mM Tris–HCl, pH 7.4, containing 1% NP-40, 0.1% SDS, 0.1 mM phenylmethylsulfonyl fluoride, and 0.15 M NaCl and then centrifuged at 12,000 *g* for 20 min to remove undissolved materials. Aliquots (20 μL) from solubilized samples were subjected to SDS/10% PAGE. The gels were impregnated with ENHANCE (Du Pont-New England Nuclear) and autoradiographed on X-ray film (Fuji RX). The absorbance at 460 nm of bands corresponding to a molecular weight of 31,000 was quantified by scanning the autoradiograms with a densitometer (Shimadzu CS-900).

Two-dimensional polyacrylamide gel electrophoresis. Two-dimensional polyacrylamide gel electrophoresis was performed according to the method of O'Farrell [22]. Cell pellets were lysed by sonication in 50 μL of 9.5 M urea/2% NP-40/5% 2-mercaptoethanol/2% Pharmalyte (pH 3–10). Isoelectric focusing in the first dimension was carried out successively at 200 V for 90 min, at 300 V for 60 min, and at 500 V for 15 hr. SDS–PAGE in the second dimension was performed in a 10% polyacrylamide gel.

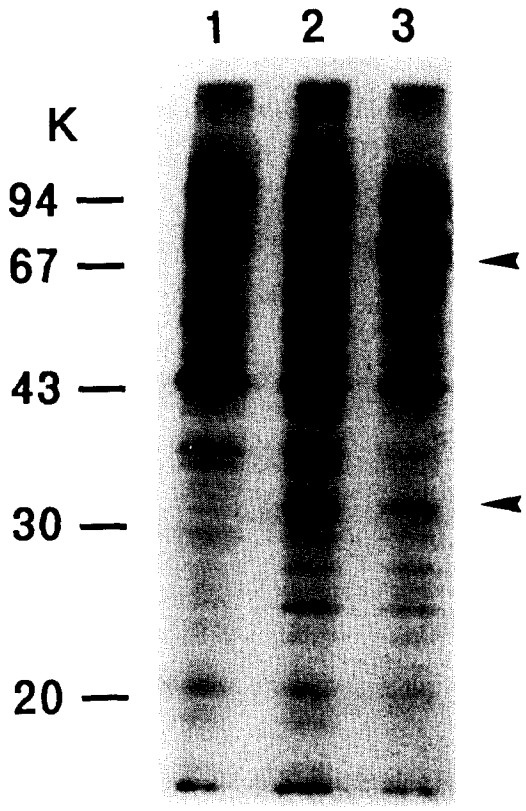

Fig. 1. Effects of PGD_2 and PGJ_2 on protein synthesis in PAEC. PAEC were treated for 6 hr with the vehicle (1), 10 μg/mL PGD_2 (2), and 10 μg/mL PGJ_2 (3) and for the following 3 hr with the respective agent in the presence of [^{35}S]methionine. The cells were lysed, and an aliquot (20 μL) was used for SDS/10% PAGE followed by autoradiography as described under Materials and Methods. The positions of molecular weight markers are shown on the left. The arrows mark the positions of p31 and p67.

RESULTS

Effects of PGD_2 and PGJ_2 on protein synthesis in PAEC. We examined the effects of PGD_2 and PGJ_2 on protein synthesis in PAEC. As shown in Fig. 1, exposure of PAEC to PGD_2 (10 μg/mL) for 9 hr induced preferential synthesis of p31. Exposure to PGJ_2 (10 μg/mL) for 9 hr not only induced the synthesis of p31 but also strongly induced the synthesis of p67, which is well known to be a heat shock protein induced by PGJ_2 in HeLa S3 cells [13]. Δ^{12}-PGJ_2 was also a strong inducer of p31 synthesis as well as of p67 synthesis, but was highly cytotoxic and markedly suppressed total protein synthesis (data not shown). To reveal whether the induction of the p31 synthesis occurs at the transcriptional level, we examined the effect of cycloheximide or actinomycin D on p31 synthesis. As shown in Fig. 2, both cycloheximide and

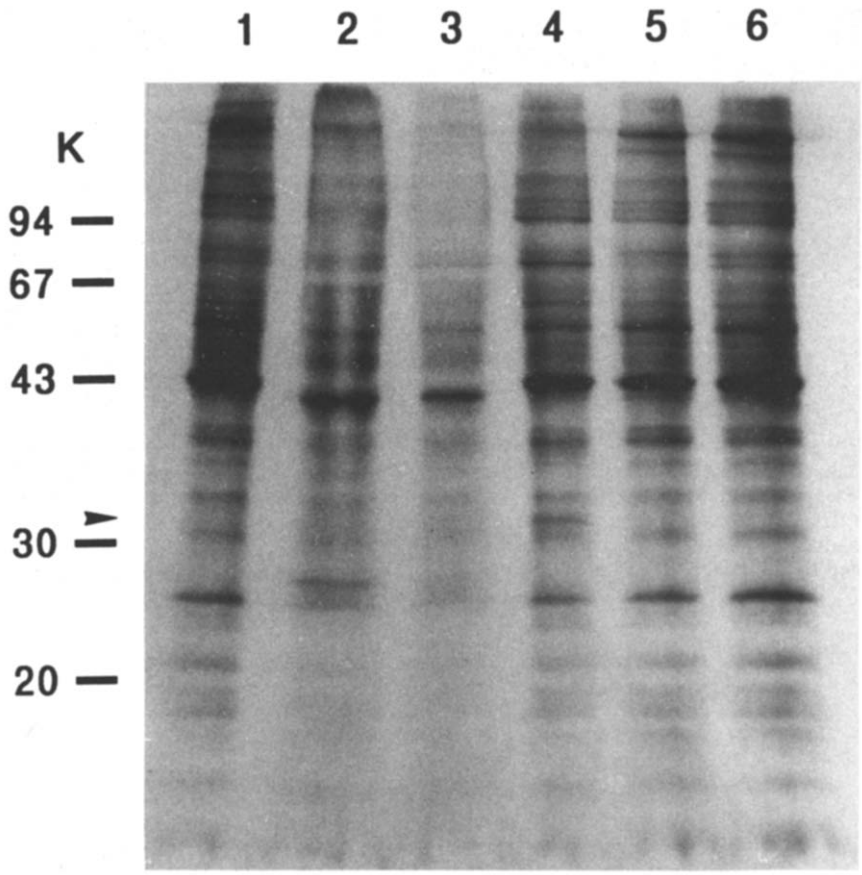

Fig. 2. Effect of cycloheximide or actinomycin D on p31 synthesis induced by PGD_2 in PAEC. Cells were treated for 6 hr with the vehicle (1), 5 μg/mL cycloheximide (2), 10 μg/mL PGD_2 and 5 μg/mL cycloheximide (3), 10 μg/mL PGD_2 (4), 10 μg/mL PGD_2 and 0.1 μg/mL actinomycin D (5), or 0.1 μg/mL actinomycin D (6), and for the following 3 hr with the respective agents in the presence of [^{35}S]-methionine. The cells were lysed and electrophoresed on SDS/10% PAGE followed by autoradiography as described under Materials and Methods. The arrow marks the position of p31.

actinomycin D almost completely inhibited p31 synthesis induced by PGD_2. The induction of p67 synthesis by PGJ_2 has been reported and its relevance to PGJ_2-induced inhibition of cell growth already examined. However, the induction and nature of p31 synthesis by PGD_2 and PGJ_2 have remained unknown. Therefore, we attempted to characterize the induction of p31 synthesis by PGD_2 and PGJ_2. Figure 3A shows the time courses of the induction of p31 synthesis by PGD_2 and PGJ_2 at 10 μg/mL. PGJ_2 markedly induced the synthesis of p31 to a maximum at 6 hr, and the level remained elevated over the basal level for over 24 hr after stimulation. In contrast, PGD_2 gradually increased the induction of p31 synthesis over a 24-hr incubation period after a 6-hr lag period, and the level of induction of p31 synthesis by PGD_2 was lower than that by PGJ_2 at 24 hr. Figure 3B shows the concentration dependencies of PGD_2 and PGJ_2 in the induction of p31 synthesis. PGJ_2 caused induction of p31 synthesis at 1 μg/mL and was maximal at 10 μg/mL. However, induction was not observed at 30 μg/mL. On the other hand, synthesis of p31 began to increase at 10 μg/mL PGD_2 and progressively increased up to 30 μg/mL. The potency of PGD_2 in the induction of p31 synthesis was one order of magnitude less than that of PGJ_2. From time courses and concentration dependencies of PGD_2 and PGJ_2, it is inferred that PGD_2 exerts its stimulatory effect on the induction of p31 synthesis through the dehydrated metabolite, PGJ_2.

To compare the effects of various PGs on p31 synthesis, cells were exposed to various PGs (10 μg/mL) for 9 hr (Fig. 4). Among the various PGs tested, PGD_2, PGJ_2, PGA_1 and 16,16-dimethyl PGD_2 strongly induced p31 synthesis. Furthermore, an enantiomer of 16,16-dimethyl PGD_2 also had induction ability. However, the E, F, and I series of PGs and PGA_2 had no induction ability. ZK110841 and BW245C are reportedly agonists of PGD_2 receptors on human platelets [23, 24], and AH6809 is an antagonist of these receptors [25]. Therefore, we investigated whether PGD_2 stimulated p31 synthesis via the activation of the platelet-type PGD_2 receptor, using these compounds. As shown in Fig. 4B, neither ZK110841 nor BW245C induced p31 synthesis, and AH6809 did not inhibit PGD_2-induced p31 synthesis, indicating that the induction of p31

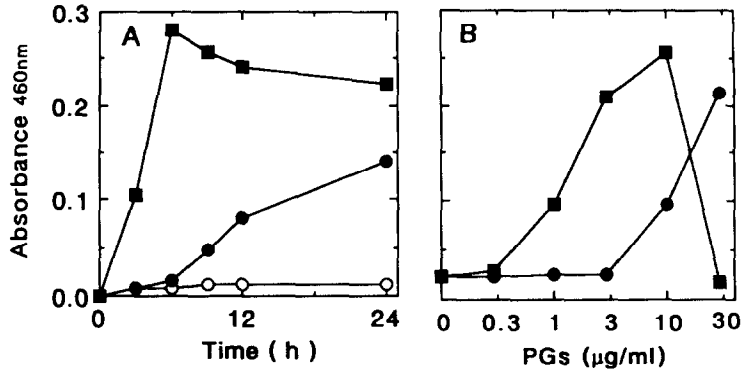

Fig. 3. Time courses and concentration dependencies of the induction of p31 synthesis by PGD_2 and PGJ_2 in PAEC. (A) Cells were treated with the vehicle (○), 10 μg/mL PGD_2 (●), or 10 μg/mL PGJ_2 (■) for the indicated times, but were incubated with the respective agent in the presence of [^{35}S]-methionine for the last 3 hr. (B) Cells were treated for 6 hr with the indicated concentrations of PGD_2 (●) or PGJ_2 (■), and for the following 3 hr with the respective agent in the presence of [^{35}S]methionine. Cell lysates were electrophoresed on SDS/10% PAGE followed by autoradiography. Ordinates show the absorbance at 460 nm of the band corresponding to a molecular weight of 31,000. Values were calculated by subtracting the absorbance at 460 nm of the band corresponding to a molecular weight of 31,000 in control cells incubated with [^{35}S]methionine for 3 hr.

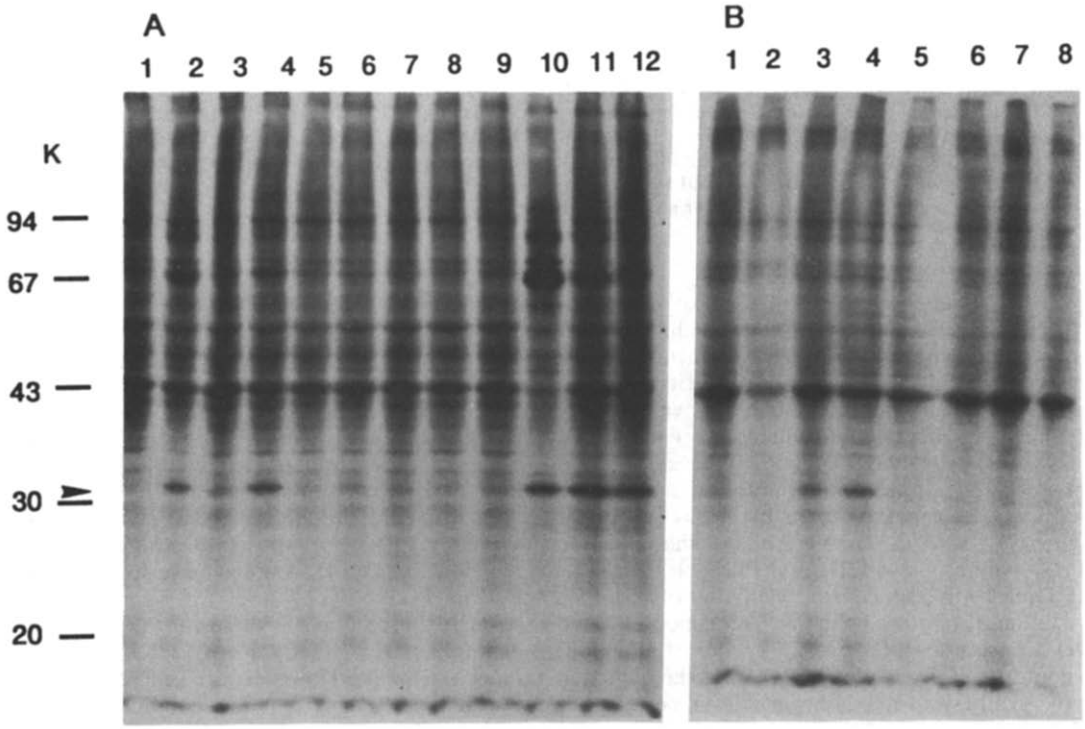

Fig. 4. Effects of various PGs on induction of p31 synthesis. Cells were treated for 6 hr; (A) with the vehicle (1), PGA_1 (2), PGA_2 (3), PGD_2 (4), PGE_1 (5), PGE_2 (6), 6-keto $PGF_{1\alpha}$ (7), $PGF_{2\alpha}$ (8), PGI_2-methyl ester (9), PGJ_2 (10), 16,16-dimethyl PGD_2 (11), or enantiomer of 16,16-dimethyl PGD_2 (12) at 10 μg/mL; (B) with the vehicle (1), 100 μM AH6809 (2), 10 μg/mL PGD_2 (3), 10 μg/mL PGD_2 and 100 μM AH6809 (4), 10 μg/mL ZK110841 (5), 10 μg/mL ZK110841 and 100 μM AH6809 (6), 10 μg/mL BW245C (7), 10 μg/mL BW245C and 100 μM AH6809 (8); and for the following 3 hr with the respective agents in the presence of [^{35}S]methionine. The cells were lysed and used for SDS/10% PAGE followed by autoradiography as described under Materials and Methods. The arrow marks the position of p31.

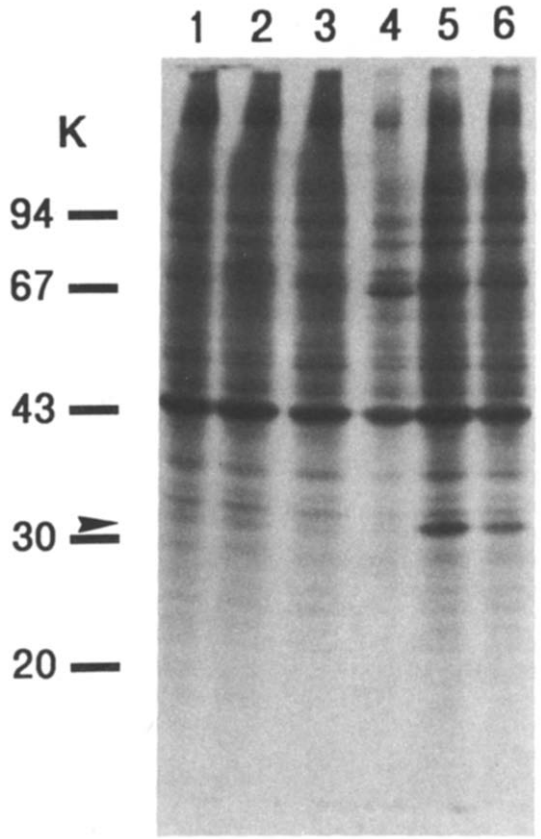

Fig. 5. Effects of various inducers of stress proteins on p31 synthesis in PEAC. Cells were treated with the vehicle (1), 100 μM TPA (2), 1 unit/mL PDGF (3), 10 μM arsenite (5), 100 μM diethylmaleate (6), for 3 hr, or cells were incubated for 60 min at 43° (4). Cells were further incubated for 1 hr with respective agents in the presence of [^{35}S]-methionine. The cells were lysed and electrophoresed on SDS/10% PAGE followed by autoradiography as described under Materials and Methods. The arrow marks the position of p31.

synthesis by PGD_2 is not mediated by the PGD_2 receptor.

Effects of various inducers of stress proteins on p31 synthesis in PAEC. PGD_2 or PGJ_2-induced p31 may belong to a family of stress proteins including heat shock proteins. Among various stress proteins, synthesis of small molecular mass stress proteins with molecular weights of around 31 kD is reportedly induced by thiol-reactive agents, heavy metals such as arsenite, PDGF, or TPA [26–28]. Thus, we examined whether high temperature, the thiol-reactive agent diethylmaleate, heavy metal arsenite, PDGF, or TPA could induce p31 synthesis. As shown in Fig. 5, 100 μM diethylmaleate or 10 μM sodium aresenite preferentially enhanced the synthesis of p31. On the other hand, enhancement of p31 synthesis was not observed in TPA- or PDGF-challenged cells. Furthermore, high-temperature treatment of cells by incubation at 43° for 60 min failed to increase the synthesis of this protein. From its induction by diethylmaleate and arsenite but not by high temperature, it appears that p31 is a member of the thiol-reactive agent-inducible group of stress proteins but is not a heat shock protein. Furthermore, we determined whether the PGJ_2-induced p31 is identical to that of arsenite. To achieve this, we compared p31 induced by PGJ_2 with the 31-kD protein induced by arsenite using two-dimensional polyacrylamide gel electrophoresis. PGJ_2-induced p31 had an isoelectric point of 5.4, similar to that of arsenite-induced p31 shown in Fig. 6. When [^{35}S]-methionine-labeled lysates from PGJ_2- or arsenite-treated cells were mixed and analyzed on the same gel, PGJ_2-induced p31 and arsenite-induced p31 exactly overlapped each other (Fig. 6d). These results suggest that PGJ_2-induced p31 is identical to arsenite-induced p31.

One of the arsenite-induced stress proteins is associated with nuclei [28]. Thus, we studied the subcellular localization of p31. The profiles for the activities of marker enzymes and DNA content in each subcellular fraction, and that for PGJ_2-induced p31 in each fraction are shown on the right and left sides of Fig. 7, respectively. PGJ_2-induced p31 was detected in nuclear, mitochondrial and microsomal fractions and the profile of the p31 distribution was not well correlated with that of each marker enzyme or DNA content. In contrast, PGJ_2-induced p67 was located mainly in the 100,000 *g* supernatant. These results suggest that PGJ_2-induced p31 is not a protein strictly located in nuclei, mitochondria or microsomes.

Comparison of PGJ_2- and PGD_2-induced p31 synthesis in several normal cells. We then tested the PGD_2 and PGJ_2 responsiveness of other normal cells in terms of the induction of p31 synthesis. As presented in Fig. 8, rat capillary endothelial cells, rat skin fibroblasts, and rat hepatocytes were responsive to both PGD_2 and PGJ_2. These results suggest that p31 may be ubiquitously induced by PGD_2 and PGJ_2 in many mammalian cells.

DISCUSSION

In the present study, we demonstrated that both PGD_2 and PGJ_2 induced p31 synthesis in PAEC. The antitumor effect of PGD_2 is now explained by the action of PGJ_2 or Δ^{12}-PGJ_2, nonenzymatic metabolites of PGD_2, but not by PGD_2 itself [7, 8]. In aqueous solution, 30–40% of PGD_2 is dehydrated to PGJ_2 after 12 hr at 37° [29]. Therefore, it is quite plausible that the stimulatory effect of PGD_2 on p31 synthesis is mediated by PGJ_2 converted from the parent compound, PGD_2. Supporting results are the following: (1) The time course of the p31 synthesis induced by PGD_2 had an approximate 6-hr lag period and the rate of this induction was lower than that of PGJ_2 (Fig. 3A); (2) the concentration–response curve of PGD_2 for the induction of p31 synthesis shifted roughly one order of magnitude toward the right from that of PGJ_2 (Fig. 3B); and (3) metabolically stable PGD_2 analogues, ZK110841 and BW245C, which have as potent antiaggregatory activities as PGD_2 in platelets, did not induce p31 synthesis, and an antagonist of PGD_2 in platelets, AH6809, did not affect the induction of p31 synthesis by PGD_2 (Fig. 4B). Among the various PGs tested,

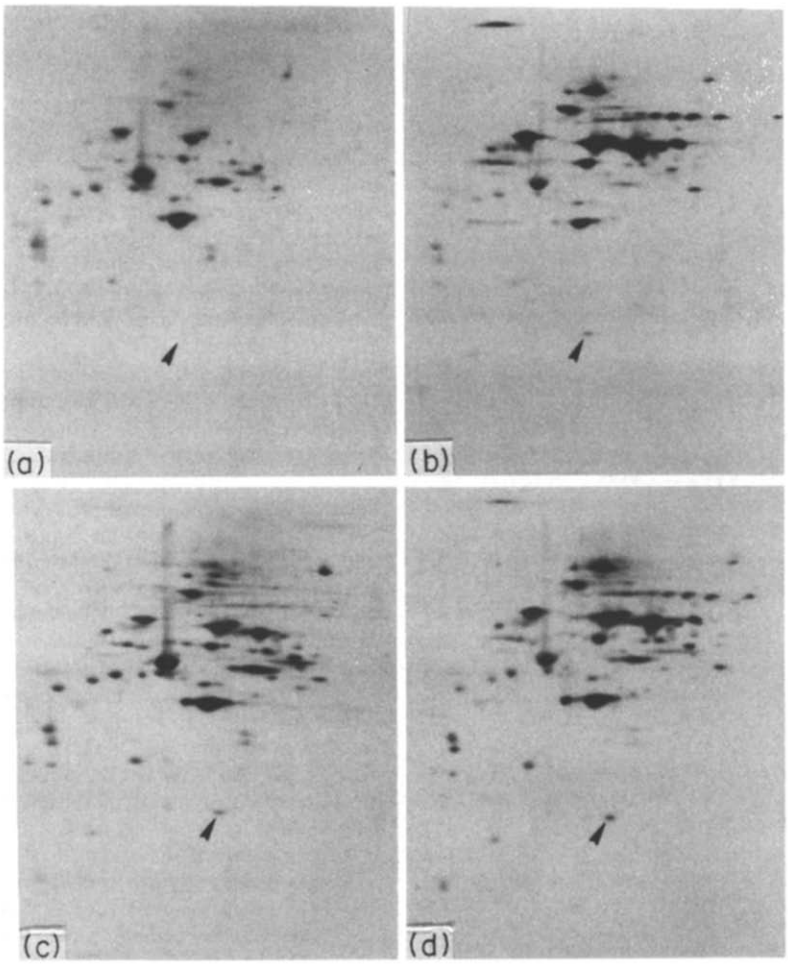

Fig. 6. Comparison of PGJ_2- or arsenite-induced protein using two-dimensional polyacrylamide gel electrophoresis. Cells were treated for 6 hr with or without 10 μg/mL PGJ_2 or 10 μM arsenite, and for the following 3 hr with or without respective agents in the presence of [^{35}S]methionine. Aliquots (20 μL) of cell lysates treated without (a) or with PGJ_2 (b), arsenite (c), or of the mixture of lysates from PGJ_2-treated and arsenite-treated cells (d) were subjected to two-dimensional polyacrylamide gel electrophoresis as described under Materials and Methods. The arrow marks the position of p31.

the induction of p31 synthesis was specific to PGJ_2, PGD_2 and its analogues (Fig. 4A). PGA_1 also induced p31 synthesis; however, PGA_2 did not. PGJ_2 appears to be a much more potent inducer of p31 synthesis than PGA_1. A series of PGs, namely dehydrates of the E series, and type J PGs are the most active in induction of 70-kD heat-shock proteins associated with inhibition of cell proliferation, type J PGs being a more potent inducer of the 70-kD proteins and more cytotoxic [30]. Meanwhile, the J and A series of PGs, characterized by the presence of an α,β-unsaturated carbonyl group in the cyclopentane ring and mutually termed cyclopentenone PGs, are the most active in the induction of p31 synthesis. Good correlation was observed between the specificities of PGs for the induction of p31 synthesis shown in Fig. 4A and for the induction of the 70-kD heat shock proteins associated with the inhibition of cell proliferation, reported by other groups [13, 30].

It was observed recently that Δ^{12}-PGJ_2 and PGA_2 enhanced the syntheses of two 68-kD proteins with isoelectric points of 5.5 and 5.6 in HeLa S3 cells, and that these proteins belong to a family of heat shock proteins [13]. From the evidence that the induction of p31 synthesis is specific for cyclopentenone PGs, it is assumed that p31 would also be one of the stress proteins including heat shock proteins. It has been reported that exposure of various types of cells to heat shock and other environmental stresses such as heavy metals results in the selective induction of proteins with molecular masses ranging from 22 to 110 kD [31, 32]. In contrast to the apparent consistent induction of higher molecular mass-stress proteins (70–90 kD) by heat shock or cyclopentenone PGs, several studies have noted the induction of small molecular mass stress proteins with molecular weights of around 30 kD. Proteins of 32 and 34 kD molecular weight are reportedly induced by heavy metals such as

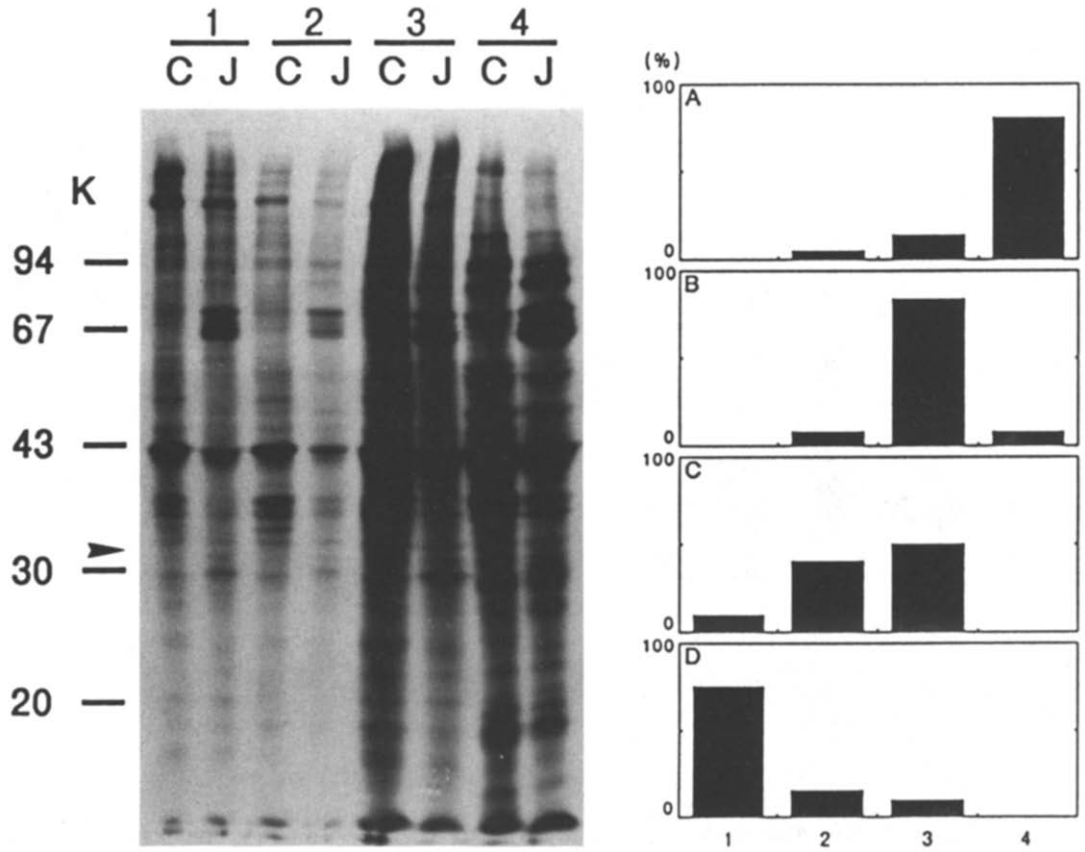

Fig. 7. Subcellular localization of p31 induced by PGJ_2 in PAEC. Cells were treated for 6 hr with the vehicle (C) or 10 μg/mL PGJ_2 (J), and for the following 3 hr with the respective agent in the presence of [^{35}S]methionine. The cells treated were fractionated and the 800 *g* pellet (1), the 800–9500 *g* pellet (2), the 9500–100,000 *g* pellet (3) or the 100,000 *g* supernatant (4) were electrophoresed on SDS/10% PAGE followed by autoradiography as described under Materials and Methods. The arrow marks the position of p31 (left side). The activities of lactate dehydrogenase (A), NADPH–cytochrome *c* reductase (B), succinate dehydrogenase (C) and DNA content (D) of each fraction (1–4) were measured as described under Materials and Methods (right side). All values are expressed as a percentage of the corresponding activity or content of the total homogenate.

sodium arsenite, or thiol-reactive agents in several cell lines including human and murine melanoma cell lines [26, 27]. In BALB/c 3T3 cells, PDGF and TPA stimulated the synthesis of a 31-kD nuclear protein with an isoelectric point of 6.8 which is also induced by arsenite [28]. The 32-kD heat shock protein induced by TPA or metal salts in BALB/c 3T3 cells has been identified as heme oxygenase with an isoelectric point of 6.4 which is present in microsomes [33]. Subcellular fractionation shows that p31 apparently spreads over nuclear, mitochondrial and microsomal fractions (Fig. 7), presenting two possible explanations for the p31 localization. One is association of p31 with other structures, and the other is widespread distribution of p31 over three of those organelles. However, definitive localization will require immunostaining of whole cells with specific antiserum. Although the isoelectric point (5.4) of p31 induced by PGJ_2 in PAEC (Fig. 6) was different from those of the stress proteins mentioned above, the isoelectric point difference could arise from either an experimental factor or a species difference. Purification and further characterization of p31 are needed for the contention that p31 is not identical with other stress proteins including heme oxygenase.

Whereas high-temperature treatment, PDGF, and TPA had no ability to induce p31 synthesis, arsenite and diethylmaleate could induce it, and it is attested by two-dimensional gel electrophoresis that p31 induced by PGJ_2 is identical to that induced by arsenite. These results provide strong evidence that p31 induced by PGJ_2 represents one of the stress proteins whose expression is primarily regulated by thiol-reactive compounds but not by hyperthermia. Diethylmaleate forms a thioester conjugate with glutathione [34]. Arsenite is also a thiol binding molecule and can possibly bind to a sulfhydryl group of glutathione to form a metal–glutathione complex. Moreover, it has been proposed that the formation of the glutathione conjugate with these thiol binding molecules may be involved in the induction of stress proteins [35, 36]. Recently, it has been reported that Δ^{12}-PGJ_2 readily conjugates with glutathione *in vitro*

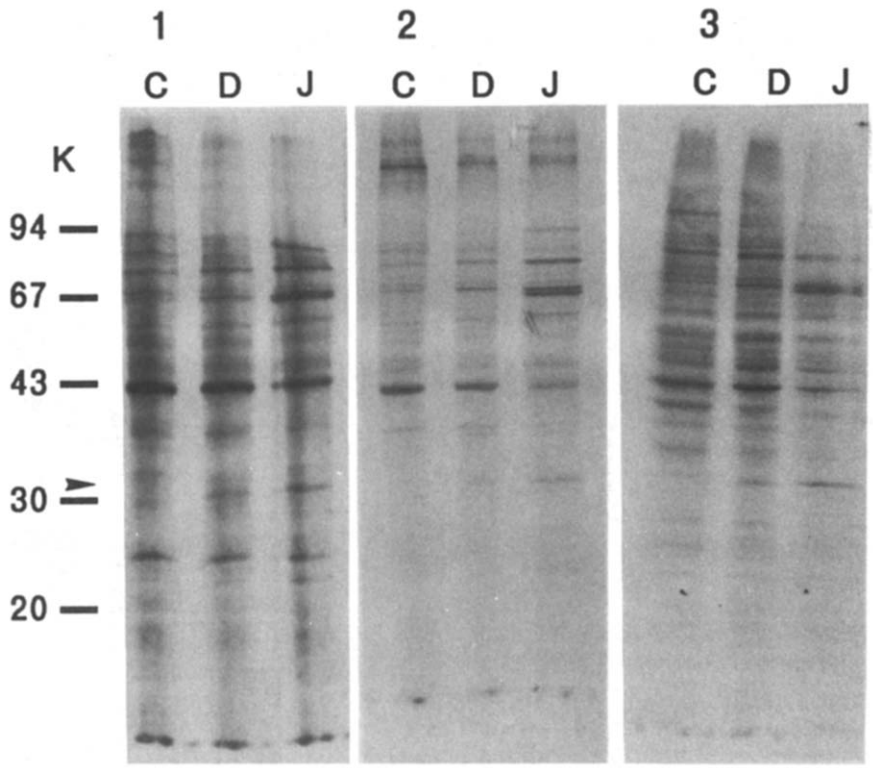

Fig. 8. Induction of p31 synthesis by PGD_2 and PGJ_2 in several types of normal cells. Rat capillary endothelial cells (1), rat fibroblasts (2), or rat hepatocytes (3) were treated for 6 hr with the vehicle (C), 10 μg/mL PGD_2 (D), or 10 μg/mL PGJ_2 (J), and for the following 3 hr with the respective agent in the presence of [^{35}S]methionine. The cells were lysed and electrophoresed on SDS/10% PAGE followed by autoradiography as described under Materials and Methods. The arrow marks the position of p31.

[37], and PGJ_2 was recovered in cell lysate primarily as a glutathione conjugate when PGJ_2 was incubated with Chinese hamster ovary or hepatoma tissue culture cells, leading to the inhibition of cell proliferation [38]. In view of the fact that glutathione is ubiquitous and present in most cells, it is inferred that cyclopentenone PGs may stimulate p31 synthesis in PAEC through conjugates of these PGs with glutathione.

The induction of 70-kD heat shock protein synthesis by cyclopentenone PGs has been observed in various tumor cell lines, but the induction of p31 synthesis by these PGs was undetectable [13, 31]. In contrast, several normal cells, such as fibroblasts, hepatocytes as well as endothelial cells, were responsive to PGJ_2 in the induction of p31 synthesis, as shown in Fig. 8. From these results, it is suggested that normal cells respond to cyclopentenone PGs but that tumor cells lose this responsiveness, suggesting that p31 induced by PGJ_2 plays a role in the metabolic regulation of normal cells. However, the biochemical role of p31 and its structural and functional relationships to the other stress proteins such as p67 have yet to be established.

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REFERENCES

1. Giles H and Leff P, The biology and pharmacology of PGD_2. *Prostaglandins* **35**: 277–300, 1988.
2. Ito S, Narumiya S and Hayaishi O, Prostaglandin D_2: A biochemical perspective. *Prostaglandins Leukot Essent Fatty Acids* **37**: 219–234, 1989.
3. Fitzpatrick FA and Wynalda MA, Albumin-catalyzed metabolism of prostaglandin D_2. *J Biol Chem* **258**: 11713–11718, 1983.
4. Fukushima M, Kato T, Ueda R, Ota K, Narumiya S and Hayaishi O, Prostaglandin D_2, a potential antineoplastic agent. *Biochem Biophys Res Commun* **105**: 956–964, 1982.
5. Simmet T and Jaffe BM, Inhibition of B-16 melanoma growth *in vitro* by prostaglandin D_2. *Prostaglandins* **25**: 47–54, 1983.
6. Sakai T, Yamaguchi N, Shiroko Y, Sekiguchi M, Fujii G and Nishino H, Prostaglandin D_2 inhibits the proliferation of human malignant tumor cells. *Prostaglandins* **27**: 17–26, 1984.
7. 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.
8. Fukushima M, Kato T, Ota K, Arai Y, Narumiya S and Hayaishi O, 9-Deoxy-Δ^9-prostaglandin D_2, a prostaglandin D_2 derivative with potent antineoplastic

and weak smooth muscle contracting activities. *Biochem Biophys Res Commun* **109**: 626–633, 1982.

9. 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**: 506–511, 1986.
10. Shimizu Y, Todo S and Imashuku S, Cycloheximide reduces PGD_2 or Δ^{12}-PGJ_2 cytotoxicity on NCG cells. *Prostaglandins* **32**: 517–525, 1986.
11. Santoro MG, Crisari A, Benedetto A and Amici C, Modulation of the growth of a human erythroleukemic cell line (K562) by prostaglandins: Antiproliferative action of prostaglandin A. *Cancer Res* **46**: 6073–6077, 1986.
12. Shimizu Y, Todo S and Imashuku S, Selective synthesis and retention of 66K protein in a human neuroblastoma cell line (NCG) treated with a cytotoxic dosage of Δ^{12}-prostaglandin J_2. *Prostaglandins* **34**: 769–781, 1987.
13. Ohno K, Fukushima M, Fujiwara M and Narumiya S, Induction of 68,000-dalton heat shock proteins by cyclopentenone prostaglandins. Its association with prostaglandin-induced G_1 block in cell cycle progression. *J Biol Chem* **263**: 19764–19770, 1988.
14. 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.
15. Björntorp P, Hansson GK, Jonasson L, Pettersson P and Sypniewska G, Isolation and characterization of endothelial cells from the epididymal fat pad of the rat. *J Lipid Res* **24**: 105–112, 1983.
16. Moldéus P, Högberg J and Orrenius S, Isolation and use of liver cells. *Methods Enzymol* **52**: 60–71, 1978.
17. Hunter E, Preparation and culture of chicken embryo fibroblasts. *Methods Enzymol* **58**: 381–384, 1979.
18. Pennington RJ, Biochemistry of dystrophic muscle. *Biochem J* **80**: 649–654, 1961.
19. Omura T and Takesue S, A new method for simultaneous purification of cytochrome b_5 and NADPH-cytochrome *c* reductase from rat liver microsomes. *J Biochem (Tokyo)* **67**: 249–257, 1970.
20. Kornberg A, Lactic dehydrogenase of muscle. *Methods Enzymol* **1**: 441–443, 1955.
21. Burton K, A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* **62**: 315–323, 1956.
22. O'Farrell PH, High resolution two-dimensional electrophoresis of protein. *J Biol Chem* **250**: 4007–4021, 1975.
23. Thierauch K-H, Stürzebecher C-ST, Schillinger E, Rehwinkel H, Radüchel B, Skuballa W and Vorbrüggen H, Stable 9β- or 11α-halogen-15-cyclohexyl-prostaglandins with high affinity to the PGD_2-receptor. *Prostaglandins* **35**: 855–868, 1988.
24. Whittle BJR, Moncada S, Mullane K and Vane JR, Platelet and cardiovascular activity of the hydantoin BW245C, a potent prostaglandin analogue. *Prostaglandins* **25**: 205–223, 1983.
25. Keery RJ and Lumley P, AH6809, a prostaglandin DP-receptor blocking drug on human platelets. *Br J Pharmacol* **94**: 745–754, 1988.
26. Shelton KR, Todd JM and Egle PM, The induction of stress-related proteins by lead. *J Biol Chem* **261**: 1935–1940, 1986.
27. Caltabiano MM, Koestler TP, Poste G and Greig RG, Induction of 32- and 34-kDa stress proteins by sodium arsenite, heavy metals, and thiol-reactive agents. *J Biol Chem* **261**: 13381–13386, 1986.
28. Disa S, Manilla AC and Scher CD, Purification and characterization of a platelet-derived growth factor and heavy metal-modulated nuclear protein. *J Biol Chem* **264**: 15993–15999, 1989.
29. Ito S, Tanaka T, Hayashi H and Hayaishi O, Problems in production of prostaglandin D_2-specific antibody. *Eicosanoids* **1**: 111–117, 1988.
30. Santoro MG, Garaci E and Amici C, Prostaglandins with antiproliferative activity induce the synthesis of a heat shock protein in human cells. *Proc Natl Acad Sci USA* **86**: 8407–8411, 1989.
31. Schlesinger MJ, Ashburner M and Tissieres A (Eds.), *Heat Shock: From Bacteria to Man*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982.
32. Atkinson BG and Walden DB (Eds.), *Changes in Eukaryotic Gene Expression in Response to Environmental Stress*. Academic Press, New York, 1985.
33. Kageyama H, Hiwasa T, Tokunaga K and Sakiyama S, Isolation and characterization of a complementary DNA clone for a M_r 32,000 protein which is induced with tumor promoters in BALB/c 3T3 cells. *Cancer Res* **48**: 4795–4798, 1988.
34. Ormstad K, Jones DP and Orrenius S, Characteristics of glutathione biosynthesis by freshly isolated rat kidney cells. *J Biol Chem* **255**: 175–181, 1980.
35. Shelton KR, Egle PM and Todd JM, Evidence that glutathione participates in the induction of a stress protein. *Biochem Biophys Res Commun* **134**: 492–498, 1986.
36. Freeman ML and Meredith MJ, Glutathione conjugation and induction of a 32,000 dalton stress protein. *Biochem Pharmacol* **38**: 299–304, 1989.
37. 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.
38. 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.