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Activation of the Mouse Heme Oxygenase-1 Gene by 15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2 Is Mediated by the Stress Response Elements and Transcription Factor Nrf2

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ABSTRACT

The mechanism of *heme oxygenase-1 (ho-1)* gene activation by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) was examined. 15d-PGJ_2 stimulated expression of HO-1 mRNA and protein and of a mouse *ho-1* gene promoter/luciferase fusion construct (*HO15luc*) in a dose-dependent manner in mouse hepatoma (Hepa) cells. *HO15luc* expression was not effected by troglitazone, a peroxisome proliferator-activated receptor-γ (PPAR-γ) ligand, but induction by 15d-PGJ_2 was abrogated by the antioxidant *N*-acetylcysteine. The primary 15d-PGJ_2 responsive sequences were localized to a 5′ distal enhancer (E1) and identified as the stress-response element, previously shown to mediate *ho-1* activation by several agents, including heme and heavy metals. Treatment of Hepa cells with 15d-PGJ_2 stimulated stress-response element-binding activity as judged by electrophoretic mobility shift assays. Antibody "supershift" experiments identified NF-E2 related factor 2 (Nrf2), but not Fos, Jun, or activating transcription factor/cyclic AMP response element binding protein transcription factors, within the 15d-PGJ_2-induced complexes. Similarly, a dominant-negative mutant of Nrf2, but not of c-Jun or c-Fos, abrogated 15d-PGJ_2-stimulated E1 transcription activity. Finally, prior induction of HO-1 in RAW264.7 mouse macrophages by 15d-PGJ_2 attenuated cell death caused by diesel exhaust particle extracts. These results demonstrate that induction of mouse HO-1 expression by 15d-PGJ_2 is independent of PPAR-γ but dependent on oxidative stress, is regulated by the oxidative stress-activated transcription factor Nrf2, and provides cytoprotective activity. Antioxid. Redox Signal. 4, 249–257.

INTRODUCTION

PROSTAGLANDINS (PGs), oxygenated metabolites of arachidonic acid, are produced in response to a variety of extracellular stimuli and regulate cellular growth, differentiation, and homeostasis (36, 48). Conventional PGs of the D, E, F, and I classes exert their effects via stimulation of G protein-coupled, seven-transmembrane receptors. On the other hand, cyclopentenone PGs—characterized by the presence of an α,β-unsaturated ketone in the cyclopentenone ring—do not appear to bind to membrane receptors and are actively transported into cells (35), where they exert a unique spectrum of biological effects including antitumor and antiviral activities, cessation of cell growth, and stimulation of osteogenesis (11, 37). These effects of cyclopentenone PGs, such as PGA_2 and PGJ_2, can be attributed in part to their ability to stimulate expression of proteins involved in multiple cellular processes including gene transcription [p53 (30) and c-Fos (15)], cell-cycle progression [p53 and p21/WAF1/CiP (19)], and stress management [heat-shock proteins (39, 47) and heme oxygenase-1 (HO-1) (26)].

HO-1, a ubiquitous stress-responsive protein, and one of the most prominent proteins induced by Δ^{12}-PGJ_2 (26, 27), is

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a key enzyme in heme metabolism, initiating the oxidative cleavage of heme to yield carbon monoxide, iron, and biliverdin; the latter is subsequently reduced to bilirubin in a non–rate-limiting step. Evidence accumulated over the past decade suggests that HO-1 functions as a cytoprotective enzyme, largely as a consequence of the antioxidant activities of biliverdin and bilirubin and the pleiotropic effects of carbon monoxide (8, 32). The biological importance of HO-1 activity, and presumably its inducibility, are most dramatically demonstrated by the physiological abnormalities, including growth retardation, anemia, leukocytosis, and tissue iron deposition, resulting from HO-1 deficiency (42, 43, 50).

As is the case with most inducers, stimulation of HO-1 expression by Δ^{12}-PGJ_2 is presumably regulated at the level of gene transcription (27, 38), but the molecular mechanism of *ho-1* gene activation by Δ^{12}-PGJ_2 is still incompletely understood. Koizumi *et al.* (27) have localized the Δ^{12}-PGJ_2-responsive sequence of the rat *ho-1* gene to a promoter region between residues −810 and −600. They have further identified the critical sequence, CATGTG, as an element that conforms to the consensus E-box motif, CANNTG, a recognition site for a large family of basic/helix–loop–helix transcription factors. Interestingly, although the mouse and rat *ho-1* gene promoter sequences in this region are highly conserved, the corresponding mouse element, TATGTG, no longer conforms to an E-box motif. Furthermore, in our analysis of the mouse *ho-1* gene, we have been unable to identify any agent that transmits *ho-1* gene inducibility solely through this promoter region. Rather, induction by most agents is mediated by two distal enhancers, E1 and E2, located approximately 4 and 10 kbp upstream of the transcription initiation site, respectively (3, 4, 8). Taken together, these observations point to the existence of additional or alternative mechanisms for *ho-1* gene activation by PGJ_2. Data presented in this report indicate that the E1 enhancer is essential for *ho-1* gene activation by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), a metabolite of Δ^{12}-PGJ_2, and that this induction is mediated by the stress response elements (StREs) (18) and the NF-E2 related factor 2 (Nrf2) transcription factor. Additional studies indicate that prior stimulation of HO-1 expression by 15d-PGJ_2 attenuates cell death induced by diesel exhaust particle (DEP) extracts.

MATERIALS AND METHODS

Materials

PGs were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) or Calbiochem–Novabiochem Corp. (La Jolla, CA, U.S.A.). DEP extracts were prepared as previously described (16).

Plasmids

The construction of plasmids pHO15luc and its corresponding mutants [ΔE1, ΔE2, and Δ(E1+E2)] (6), pE1-luc and its corresponding mutants (1, 6), and the dominant-negative mutants (DNMs) of Nrf2 and c-Jun (5) have been described. The MafK DNM (29) and the A-Zip type mutant of c-Fos (40) were kindly provided by Drs. Stuart Orkin (Harvard University) and Chuck Vinson (NCI).

Cell culture, transfection, and enzyme assays

Mouse hepatoma (Hepa) and macrophage (RAW264.7) cells were cultured in a humidified atmosphere at 37°C in Dulbecco's modified Eagle's medium (Life Technologies Inc., Grand Island, NY, U.S.A.) containing 0.45% glucose and 10% fetal bovine serum (Mediatech, Herndon, VA, U.S.A.), with 50 μg/ml gentamycin. Stable transfections were carried out by the calcium phosphate precipitation technique as previously described (1, 2). Transient transfections were carried out either by the calcium phosphate precipitation technique or by using Effectene Transfection Reagent (Qiagen, Valencia, CA, U.S.A.) according to the manufacturer's recommendation. Transfection efficiency was monitored by co-expression of *E. coli* β-galactosidase encoded by plasmid pCMV/β-gal. Preparation of cell extract and measurement of reporter enzyme activities were carried out as described (2). Additional details are provided in the figure legends.

RNA and protein blot analyses

Total RNA was isolated by the procedure of Chomczynski and Sacchi (9), and RNA dot blot analysis was carried out as previously described (6). Hybridization signals were quantified using a Storm Phosporimager (Molecular Dynamics, Sunnyvale, CA, U.S.A.). Western blot analysis for HO-1 was carried out as previously described (14) using monoclonal or polyclonal anti-rat HO-1 antibodies (StressGen Biotech, Victoria, BC, Canada) and the ECL detection system (Amersham Pharmacia Biotechnology, Piscataway, NJ, U.S.A.).

Electrophoretic mobility shift assay (EMSA)

Hepa cells were seeded (4×10^6 cells/10-cm plate) and cultured for 40–48 h in complete medium and an additional 24 h in serum-free medium. Cells were treated with vehicle or 25 μ*M* 15d-PGJ_2 in serum-free medium for 3 h and then collected for preparation of whole-cell extracts (13). A double-stranded oligonucleotide (IDT Inc., Coraville, IA, U.S.A.) containing the sequence 5′-TTTTATGCTGTGTCATGGTT-3′ (core StRE sequence underlined) was used as probe in EMSA reactions using conditions previously reported (13). In antibody supershift assays, 1 μl of preimmune serum or anti-MafG serum (kindly provided by Dr. V. Blank, Lady Davis Institute for Medical Research, Montreal, Quebec, Canada) or 2 μg of preimmune IgG or antitranscription factor IgG (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) was added to the reaction mixture and incubated for 20 min at room temperature prior to electrophoresis.

Analysis of cell death using flow cytometry

RAW264.7 cells were plated (1×10^5 cells/well of six-well plate) and cultured for 16 h. Cells were pretreated with vehicle, 15d-PGJ_2 (0.53 μ*M*), tin protoporphyrin (SnPP, 7.5 μg/ml), or both 15d-PGJ_2 and SnPP for 48 h. The culture medium was then replaced with medium containing the same agents in the presence or absence of DEP extract (100 μg/ml), and the cells were cultured for an additional 16 h. During exposure to DEP, the 15d-PGJ_2 concentration was increased to 1.6 μ*M*. Cells were collected by scraping, washed

in phosphate-buffered saline, and stained with propidium iodide (PI, 1 μg/ml). Flow cytometric analysis was performed using a FACScan (Becton Dickinson, Mountain View, CA, U.S.A.) equipped with a 488-nm argon laser. Forward and side scatters were used to gate out cellular debris. PI fluorescence was analyzed in FL-2 channel. The data were analyzed using CellQuest software (Becton Dickinson).

RESULTS

15d-PGJ_2 stimulates the expression of HO-1 mRNA and protein in mouse Hep cells

Treatment of Hepa cells with 15d-PGJ_2 for 3 h stimulated HO-1 mRNA accumulation in a dose-dependent manner (Fig. 1A). The steady-state level of HO-1 mRNA, normalized to the amount of ribosomal protein S3 mRNA, increased ~30-fold at the highest concentration (20 μ*M*) of 15d-PGJ_2 tested. A similar accumulation of HO-1 protein was also observed after exposure to 20 μ*M* 15d-PGJ_2 (Fig. 1B).

15d-PGJ_2 stimulates ho-1 *promoter activity in Hepa cells*

Reasoning that induction of HO-1 expression by 15d-PGJ_2 is regulated at the level of gene transcription, we examined the expression of *HO15luc*, a firefly luciferase gene under the control of a 15-kbp mouse *ho-1* promoter fragment. Based on luciferase activity measurements, 15d-PGJ_2 stimulated expression of the stably integrated *HO15luc* gene in a dose-dependent manner (Fig. 1) and to a similar extent to that observed with the endogenous *ho-1* gene (Fig. 2). This response was relatively specific for 15d-PGJ_2 as no induction or only minimal induction was observed with other PGs, including PGA_1, PGA_2, PGE_1, PGE_2, and PGF_α. Unless otherwise indicated, subsequent studies were carried out at a 15d-PGJ_2 concentration of 25 μ*M*, a level at which no cytotoxicity was observed under our experimental conditions (data not shown).

15d-PGJ_2 stimulates mouse ho-1 *gene activation through a peroxisome proliferator-activated receptor-γ (PPAR-γ)-independent pathway*

15d-PGJ_2 is a natural ligand of PPAR-γ, a member of the nuclear receptor superfamily of ligand-dependent transcription factors that is also activated by antidiabetic thiazolidinediones, such as troglitazone (10). To determine if *ho-1* gene induction is mediated by PPAR-γ, we examined the effect of troglitazone on *HO15luc* expression. As shown in Fig. 3A, troglitazone, up to a concentration of 10 μ*M*, had no significant effect on luciferase activity. In addition to functioning as a PPAR-γ ligand, 15d-PGJ_2 is also known to produce cellular oxidative stress (28). To examine the role of oxidative stress-dependent signaling on *ho-1* gene activation, we measured the effect of *N*-acetylcysteine (NAC), an antioxidant and glutathione precursor, on *HO15luc* induction. Treatment of Hepa cells with varying concentrations of NAC decreased 15d-PGJ_2-induced luciferase activity in a dose-dependent manner with essentially complete inhibition observed at 4 m*M* NAC (Fig. 3B). Interestingly, under similar conditions, heme-mediated induction of *HO15luc* (Fig. 3B) was not affected by NAC. This divergent effect of NAC was also observed with induction of the endogenous *ho-1* gene by 15d-PGJ_2 and heme (data not shown).

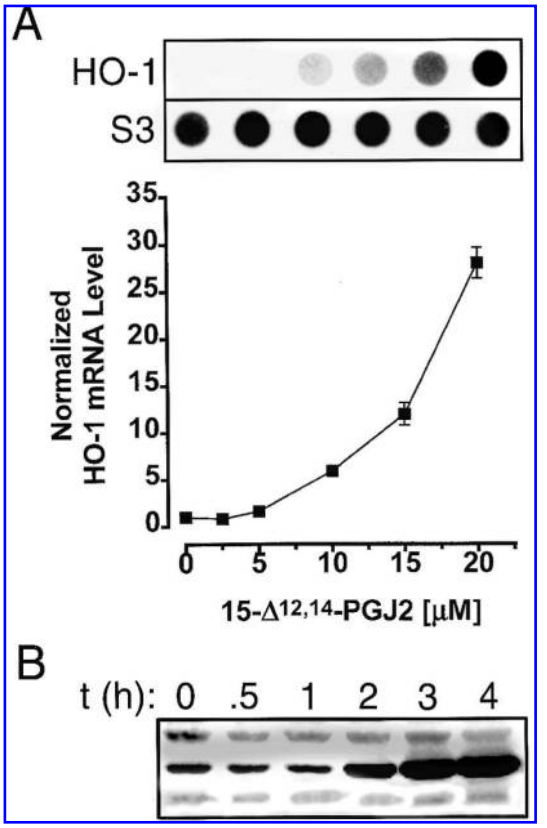

FIG. 1. 15d-PGJ_2 stimulates accumulation of HO-1 mRNA (A) and protein (B) in Hepa cells. (A) Hepa cells (5×10^5 cells/well of six-well plate) were cultured for 48 h in complete medium and for 24 h in serum-free medium and subsequently treated with the indicated concentrations of 15d-PGJ_2 for 3 h. Total RNA was isolated, and 5-μg portions were blotted onto a nylon membrane. The membrane was hybridized to a radiolabeled rat HO-1 cDNA probe, stripped, and reprobed with a radiolabeled rat ribosomal protein S3 cDNA. Signals were quantified using a phosphorimager, and relative HO-1 mRNA levels, normalized to S3 mRNA, are presented. The data represent the average and range of two independent experiments. (B) Hepa cells were cultured as in A and treated with 20 μ*M* 15d-PGJ_2 for the indicated times. Twenty-microgram portions of whole-cell extracts were electrophoresed on a 15% polyacrylamide gel, transferred to nitrocellulose membranes, and subjected to western blotting using a polyclonal anti-rat HO-1 antibody.

Identification of the StREs as the 15d-PGJ_2-responsive elements of the mouse ho-1 *gene*

Previous studies (3, 4) from our laboratory have identified two enhancer regions, E1 and E2, located approximately 4 kbp and 10 kbp upstream of the transcription initiation site, respectively, as the primary loci responsible for *ho-1* gene activation

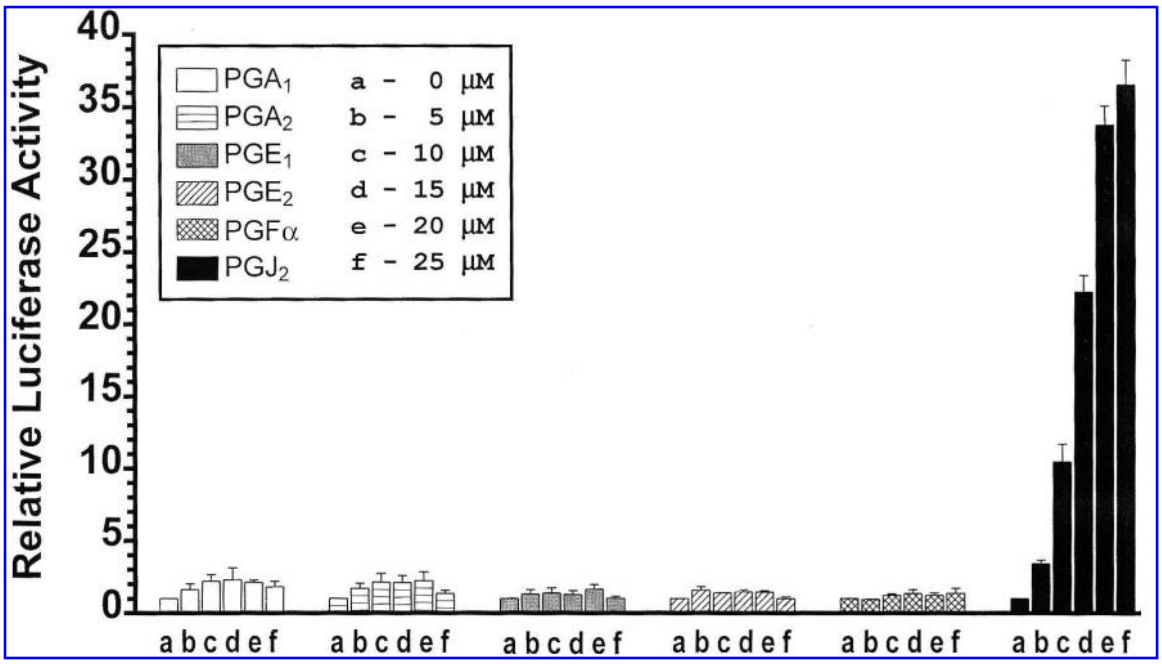

FIG. 2. 15d-PGJ$_2$ stimulates *HO15luc* expression. *HO15luc* stable transfectants of Hepa cells were plated (2×10^5 cells/well of 12-well plate), cultured as described in the legend to Fig. 1, and treated for 5 h with the indicated concentrations of the designated PGs. Aliquots representing 10% of the cell extracts were used for measurement of luciferase activity, which was normalized to protein content. Normalized activity in vehicle-treated cells was arbitrarily assigned a value of 1. Data are presented as means ± SD ($n = 3$).

in response to multiple agents, including heme and heavy metals. To determine the role of these regions in 15d-PGJ$_2$-dependent gene activation, we tested the expression of *HO15luc* mutants lacking one or both enhancers. Targeted deletion of the 161-bp E2 enhancer or a 521-bp fragment containing the 268-bp E1 enhancer reduced basal luciferase activity by ~70%, but no additional dimunition was observed when both enhancers were excised (Fig. 4A). Deletion of E1 or E2 also diminished 15d-PGJ$_2$-dependent luciferase activity, by approximately 80% and 25%, respectively, indicating a greater importance of E1 in this response. The double mutant promoter, Δ(E1+E2), was only minimally responsive to 15d-PGJ$_2$ (1.5-fold induction).

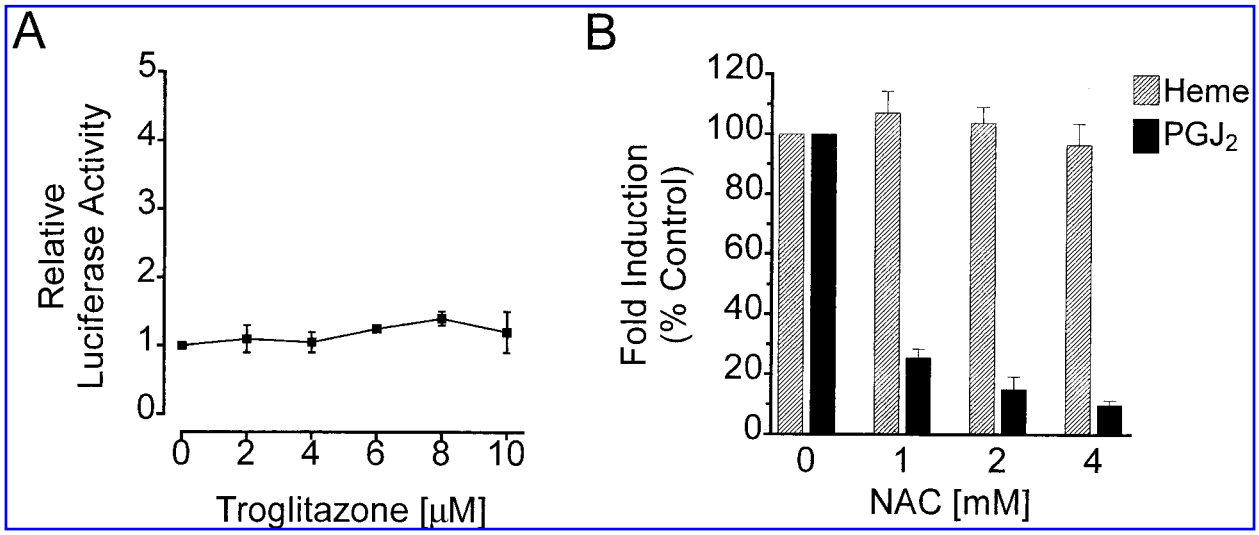

FIG. 3. Induction by 15d-PGJ$_2$ is inhibited by the antioxidant NAC. *HO15luc*-transfected Hepa cells were plated and cultured as described in the legend to Fig. 2. Cells were treated for 5 h with the indicated concentrations of troglitazone (**A**) or 25 μ*M* 15d-PGJ$_2$ or 10 μ*M* heme (**B**). NAC, at the indicated concentrations, was added 30 min prior to and during the entire incubation period. Luciferase activity was normalized to protein content, and data are presented as means ± SD ($n = 3$). For experiments depicted in B, average induction by 15d-PGJ$_2$ and heme was 28.7- and 32.7-fold above vehicle-treated cells, respectively.

Because of its apparently greater importance, the activity of E1 was examined in more detail. As shown in Fig. 4B, expression of an E1-driven luciferase reporter gene, pE1Luc, was increased >10-fold in response to 15d-PGJ_2. Among other *cis*-elements (1), the E1 enhancer contains three distinct StREs (Fig. 4B), which have been identified as the dominant inducer-responsive elements within this region (1, 6). Mutation of each of the StREs individually diminished 15d-PGJ_2-responsiveness to varying degrees; the StRE1 or StRE3 mutant (M1 and M3, respectively) exhibited 30–40% of native E1 activity, whereas mutation of StRE2 (M2) almost completely abolished inducibility. As would then be expected, the triple mutant (M123) could not be activated by 15d-PGJ_2. From these results we conclude that all three StREs contribute to 15d-PGJ_2-dependent E1 transcription activity, but that StRE2 is essential for this response.

Treatment of Hepa cells with 15d-PGJ_2 stimulates StRE2-binding activity

EMSA reactions using a StRE2 probe and whole-cell extracts from Hepa cells were carried out to identify DNA-binding proteins potentially responsible for 15d-PGJ_2-mediated *ho-1* gene induction. One prominent, specific StRE2-protein complex (Fig. 5, lane 2, "A") was detected when using extracts from vehicle [dimethyl sulfoxide (DMSO)]-treated cells. Treatment with 15d-PGJ_2 substantially increased the level of complex A (or a distinct complex comigrating with complex A) and also stimulated formation of a second complex (lane 3, "B"). Complex B was not detected in all experiments and may result from the degradation of the protein(s) in complex A. The consensus core StRE, (T/C)GCTGAGTCA, resembles the consensus binding sites for the activator protein-1 (AP-1) (Jun–Jun or Jun–Fos dimers), cyclic AMP response element binding protein (CREB)/ activating transcription factor (ATF), Maf, and Cap 'N' Collar–basic/leucine zipper (CNC-bZIP) families of transcription factors, which function as obligate dimers. "Supershift" EMSA reactions using antibodies directed against one or more members of these protein families were carried out to identify specific StRE-binding proteins (StRE-BPs) and potential 15d-PGJ_2-responsive transcription factor(s). Of several factors tested, only Nrf2, a CNC-bZIP protein, was identified in the 15d-PGJ_2-induced complex(es) (Fig. 5).

DNMs of Nrf2 and MafK attenuate gene activation by 15d-PGJ_2

Consistent with the EMSA results, overexpression of an Nrf2 DNM significantly reduced both basal and 15d-PGJ_2-stimulated pE1Luc expression to 25% and 8%, respectively, of that observed in corresponding control cells (Fig. 6). MafK, like other small Maf proteins, dimerizes most prominently with CNC-bZIP proteins (14, 17, 20, 23). A MafK DNM, which would be expected to dimerize with Nrf2 but inhibit subsequent DNA binding of the dimer (29), also diminished both basal and induced E1 activities (70% and 19%, respectively, of control levels). Conversely, DNMs of AP-1 constituents, c-Jun and c-Fos, slightly enhanced pE1Luc expression, indicating that Jun and Fos proteins may actually function as weak repressors of E1-dependent *ho-1* gene regulation.

Pretreatment with 15d-PGJ_2 protects RAW264.7 macrophages from DEP extract-induced cell death

Because HO-1 is known to possess cytoprotective activity, we tested the ability of 15d-PGJ_2-induced HO-1 to protect RAW264.7 cells from subsequent DEP extract-mediated cell death. As observed with Hepa cells, treatment of RAW264.7

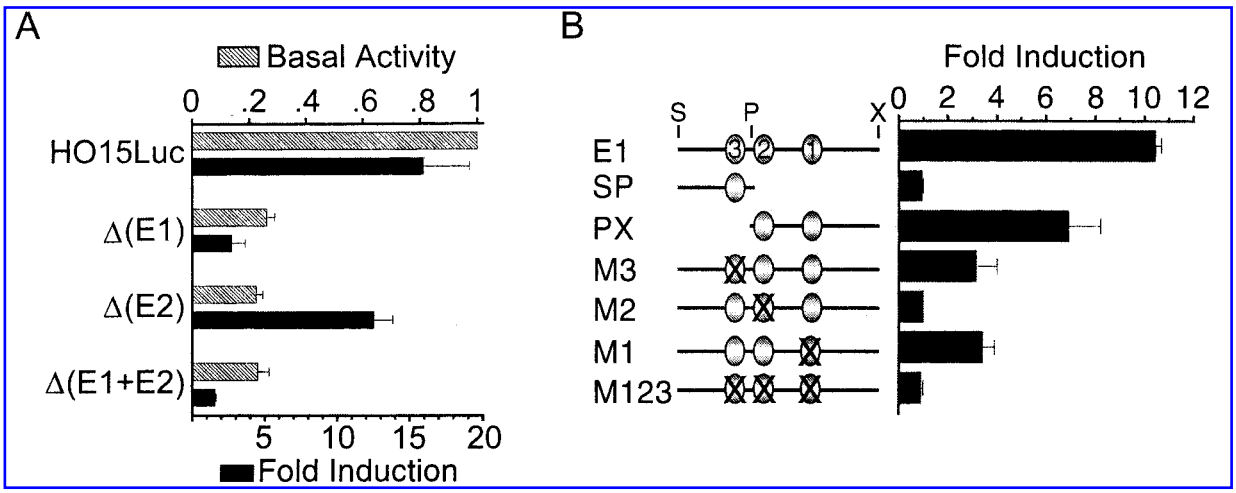

FIG. 4. Identification of StREs as 15d-PGJ_2-responsive *cis*-elements. Hepa cells were plated (5×10^5 cells/well of six-well plate) 24 h prior to transient transfection of pHO15luc and its corresponding mutants (**A**) or pE1Luc (E1) and its derivatives (**B**). Transfection was carried out for 6 h by the calcium phosphate co-precipitation method with DNA mixtures consisting of (per well) 2.5 μg of the luciferase plasmid and 2.5 μg of pCMV/β-gal. Cells were cultured overnight in complete medium and for 24 h in serum-free medium prior to treatment with vehicle or 25 μ*M* 15d-PGJ_2 in serum-free medium for 5 h. Luciferase activities were normalized with the corresponding β-galactosidase activities, and data are presented as means ± SD ($n = 3$). In B, StREs and their relative positions are indicated by numbered ovals; StRE mutations are marked by ovals with crosses. S (Sac I), P (Pvu II), and X (Xba I) represent restriction endonuclease sites in the E1 fragment.

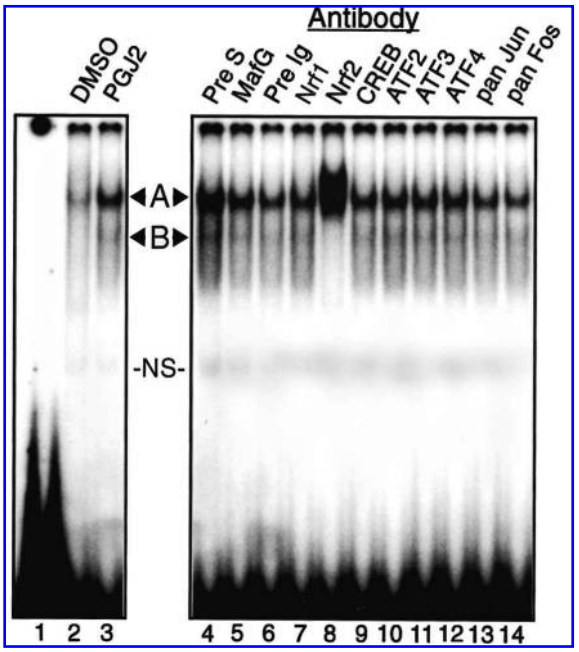

FIG. 5. 15d-PGJ$_2$ stimulates StRE2 binding activity. EMSA reactions were carried out as described in Materials and Methods using an StRE2 probe and extracts prepared from Hepa cells treated with vehicle (DMSO) or with 25 μ*M* 15d-PGJ$_2$ for 3 h. Preimmune serum ("Pre S"), preimmune IgG ("Pre Ig"), or antibodies directed against the indicated transcription factors were used in antibody supershift analysis. The gels were autoradiographed for 24 h. NS, nonspecific.

cells with 15d-PGJ$_2$ (1.6 μ*M*), but not with PGB$_2$, stimulated HO-1 protein accumulation (Fig. 7A) without appreciably affecting cell viability (Fig. 7B). DEP extract (50 μg/ml) treatment also increased the amount of HO-1 protein, but prolonged exposure to a higher concentration of DEP extract (16 h, 100 μg/ml) reduced cell viability by ~70%. Pretreatment with 15d-PGJ$_2$ reversed this cell mortality by slightly >50% (71% versus 32% dead cells). This cytoprotective effect of 15d-PGJ$_2$ was effectively blocked by cotreatment with the HO inhibitor SnPP, providing support for a role of HO-1 in the protective response. Consistent with this role, SnPP by itself reduced cell viability by 22% in this experiment, presumably by inhibiting basal HO activity.

DISCUSSION

Confirming results from previous studies (27) using Δ^{12}-PGJ$_2$, a precursor of 15d-PGJ$_2$, we show that the latter directly induces HO-1 expression in mouse liver and macrophage cell lines. Given the comparable levels of stimulation of the endogenous *ho-1* gene (Fig. 1) and the stably transfected *HO15Luc* fusion gene (Fig. 2), it appears that this induction is regulated primarily at the level of gene transcription, at least in Hepa cells. We have further characterized this mechanism by identifying the 15d-PGJ$_2$-responsive *cis*-elements and *trans*-factor: the StREs and Nrf2, respectively.

We have previously demonstrated that the StREs, in one or both of the enhancers, regulate *ho-1* induction by a diverse array of agents, including the substrate heme, 12-*O*-tetradecanoylphorbol 13-acetate, lipopolysaccharide, various electrophiles, hyperoxia, DEP extract, and heavy metals such as cadmium, zinc, mercury, and cobalt (for reviews, see 8, 13, 31). Although structurally and chemically diverse, these inducers share the ability to produce cellular oxidative stress. Inclusion of 15d-PGJ$_2$ in this group further highlights the dominant role of the StRE in inducer-dependent, mouse *ho-1* gene regulation.

The StRE resembles the binding site for several families of basic leucine zipper (bZIP) transcription factors: AP-1, CREB/ATF, Maf, and CNC-bZIP. Indeed, we have previously shown that one or more members of each of these bZIP subfamilies binds to the StRE (4, 6, 8, 13, 14). The existence of multiple StRE-BPs provides a mechanism for inducer-specific or inducer-selective regulation of the *ho-1* gene via a single type of element. Although all potential StRE-BPs were not examined in the present study, the antibody "supershift" analysis in conjunction with the DNM experiments strongly implicate Nrf2 in 15d-PGJ$_2$-mediated *ho-1* gene activation. Conversely, these experiments rule out the role of AP-1 proteins in this response as the pan-Jun and pan-Fos antibodies would recognize all members of each of their respective families. To the best of our knowledge, this is the first report demonstrating that Nrf2 is a 15d-PGJ$_2$-responsive transcription factor.

Nrf2, like Fos proteins, is incapable of forming homodimers (14, 34) and dimerizes most prominently with small

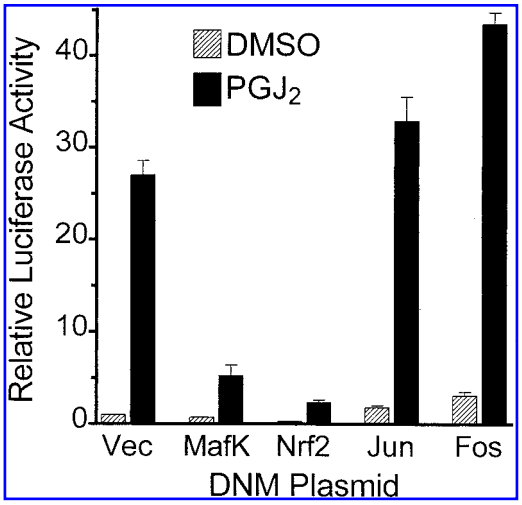

FIG. 6. DNMs of Nrf2 and MafK inhibit pE1-Luc induction by 15d-PGJ$_2$. Hepa cells were plated (2×10^5 cells/well of 12-well plate) and transfected (Effectene method) 24 h later with DNA mixtures consisting of (per well) 150 ng of pE1-Luc, 50 ng of pCMV/β-gal, and 300 ng of the empty vector ("Vec") or the indicated DNM expression plasmid. After a 24-h incubation period, cells were treated with vehicle (DMSO) or 25 μ*M* 15d-PGJ$_2$ in serum-free medium for 5 h. Luciferase activities were normalized with the corresponding β-galactosidase activities and are presented relative to the activity in vehicle-treated, vector-transfected cells. Data are means ± SD ($n = 3$).

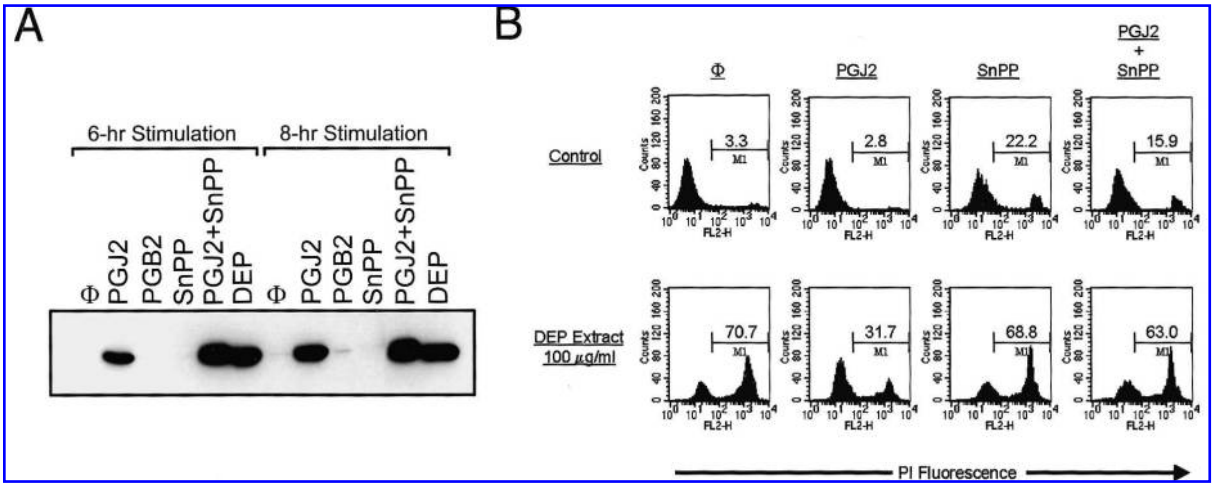

FIG. 7. Pretreatment with 15d-PGJ_2 protects RAW264.7 macrophages from cell death by DEP extract. (**A**) RAW264.7 cells were plated (2×10^6 cells/well of six-well plates), cultured for 16 h, and treated with vehicle (ϕ), 15d-PGJ_2 (PGJ2, 1.6 μ*M*), PGB2 (5 μ*M*), SnPP (7.5 μg/ml), or DEP extract (50 μg/ml) for 6 or 8 h. HO-1 protein was detected by immunoblotting. (**B**) Cell culture and treatment and flow cytometry were carried out as described in Materials and Methods. Results from a typical experiment (out of three) are presented. The percentage of cells exhibiting PI staining under each experimental condition is indicated.

Maf proteins (14, 17, 20, 33). We have recently provided evidence for a role of Nrf2–MafG heterodimers in *ho-1* gene induction by cobalt in Chinese hamster ovary cells (13), but were consistently unable to detect MafG in the 15d-PGJ_2-induced StRE2–protein complex of Hepa cells, even though this protein is sufficiently expressed in these cells (unpublished observation). It is conceivable that Nrf2 dimerizes with other small Maf proteins, such as MafK and MafF, to mediate *ho-1* gene activation. Recently, we have identified ATF4 as an Nrf2 dimerization partner (14), but ATF4 was also not detected in the StRE2–protein complex(es). Additional studies will be necessary to define more precisely the dimeric nature of the StRE-BP(s) responsible for *ho-1* gene activation in response to 15d-PGJ_2.

15d-PGJ_2 is known to regulate target gene expression by at least two general mechanisms: activation of PPAR-γ and/or activation of the stress-response pathway. The identification of StREs, as opposed to a sequence similar to the consensus PPAR-γ binding site (10, 24), as the active *cis*-elements argues that induction of *ho-1* by 15d-PGJ_2 proceeds primarily via a PPAR-γ-independent pathway. This conclusion is further strengthened by the observations that the synthetic PPAR-γ ligand troglitazone does not influence *ho-1* promoter activity, but that induction by 15d-PGJ_2 is completely abrogated by the antioxidant NAC. The insensitivity of the mouse *ho-1* promoter to troglitazone is consistent with the observations of Kasai *et al.* (23) who reported that whereas 15d-PGJ_2 and its precursors, PGJ_2 and PGD_2, readily stimulate HO-1 mRNA accumulation in human thyrocytes, troglitazone and pioglitazone, another antidiabetic thiazolidinedione, are without effect. In contrast to these results, Kitamura *et al.* (25) reported a stimulatory effect of both 15d-PGJ_2 and antidiabetic thiazolidinediones on HO-1 expression in primary rat glial cells, thus providing evidence for a role of PPAR-γ in *ho-1* gene regulation. It is conceivable that the latter pathway is operative in a cell-specific manner.

Cyclopentenone PGs have been implicated in the resolution of acute inflammatory reactions (12). Indeed, 15d-PGJ_2 inhibits the production of proinflammatory cytokines and inducible nitric oxide synthetase in activated monocytes by negatively interfering with the transcription factors (*e.g.*, AP-1, nuclear factor-κB, STAT) that regulate the corresponding genes (22, 45). Such interference could arise by either PPAR-γ dependent (45) or -independent mechanisms (46). In addition to inhibiting proinflammatory pathways, 15d-PGJ_2 could conceivably promote resolution of inflammation by activating antiinflammatory systems. HO-1 and particularly Nrf2 may be important participants in this effector arm of 15d-PGJ_2 function. For instance, in a carrageenan-inducedmodel of acute pleurisy, elevation of HO-1 expression suppresses, whereas inhibition of HO enzymatic activity potentiates, the inflammatory response (49). Furthermore, overexpression of HO-1 in RAW cells attenuates lipopolysaccharide-induced tumor necrosis factor-α production, and this inhibitory response can be mimicked by administration of exogenous carbon monoxide, which also inhibits the expression of other proinflammatory cytokines, including interleukin-1β and macrophage inflammatory protein-1β (41). Although our studies with the macrophage cells only examined the cytoprotective capability of 15d-PGJ_2-induced HO-1, it is likely that the cytoprotective, antioxidant, and antiinflammatory activities of HO-1 are not mutually exclusive properties. If this is the case, and given that accumulating evidence points to Nrf2 as an important regulator of gene-encoding proteins with antioxidant and xenobiotic detoxification functions (21), activation of Nrf2 may provide an even more significant mechanism for manifestation of the various biological effects, including antiproliferation, antitumorogenesis, and osteogenesis, of cyclopentenone PGs in general and 15d-PGJ_2 in particular. For instance, Nrf2-deficient mice exhibit a significantly higher burden of benzo[a]pyrene-induced gastric neoplasia and reduced chemoprotective efficacy than wild-

type animals (44). Furthermore, using gene array analysis, Beck *et al*. (7) have recently shown that expression of Nrf2 is up-regulated during osteoblast differentiation. Further studies will be necessary to establish more conclusively the role of Nrf2 in manifestation of the various cellular and biological effects of cyclopentenone PGs.

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ABBREVIATIONS

AP-1, activator protein-1; ATF, activating transcription factor; CNC-bZip, Cap 'N' Collar–basic/leucine zipper; CREB, cyclic AMP response element binding protein; DEP, diesel exhaust particle; DMSO, dimethyl sulfoxide; DNM, dominant-negative mutant; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; EMSA, electrophoretic mobility shift assay; HO, heme oxygenase; NAC, *N*-acetylcysteine; Nrf2, NF-E2 related factor 2; PG, prostaglandin; PI, propidium iodide; PPAR, peroxisome proliferator-activated receptor; SnPP, tin protoporphyrin IX; StRE, stress response element; StRE-BP, stress response element binding protein.

REFERENCES

1. Alam J. Multiple elements within the 5′ distal enhancer of the mouse heme oxygenase-1 gene mediate induction by heavy metals. *J Biol Chem* 269: 25049–25056, 1994.
2. Alam J. Functional analysis of the heme oxygenase-1 gene promoter. In: *Current Protocols in Toxicology*, edited by Maines MD, Costa LG, Reed DJ, Sassa S, and Sipes IG. New York, NY: John Wiley & Sons, Inc., 2000, Vol. 1, Supplement 6, unit 9.7.
3. Alam J, Cai J, and Smith A. Isolation and characterization of the mouse heme oxygenase-1 gene. Distal 5′ sequences are required for induction by heme or heavy metals. *J Biol Chem* 269: 1001–1009, 1994.
4. Alam J, Camhi S, and Choi AMK. Identification of a second region upstream of the mouse heme oxygenase-1 gene that functions as a basal level and inducer-dependent transcription enhancer. *J Biol Chem* 270: 11977–11984, 1995.
5. Alam J, Stewart D, Touchard C, Bionapally S, Choi AMK, and Cook JL. Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene. *J Biol Chem* 274: 26071–26078, 1999.
6. Alam J, Wicks C, Stewart D, Gong P, Touchard C, Otterbein S, Choi AMK, Burrow ME, and Tou J. Mechanism of heme oxygenase-1 gene activation by cadmium in MCF-7 mammary epithelial cells. *J Biol Chem* 275: 27694–27702, 2000.
7. Beck GR Jr, Zerler B, and Moran E. Gene array analysis of osteoblast differentiation. *Cell Growth Differ* 12: 61–83, 2001.
8. Choi AMK and Alam J. Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. *Am J Respir Cell Mol Biol* 15: 9–19, 1996.
9. Chomczynski P and Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal Biochem* 162: 156–159, 1987.
10. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, and Evans RM. 15-Deoxy-deltal2,14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. *Cell* 83: 803–812, 1995.
11. Fukushima M. Biological activities and mechanisms of action of PGJ2 and related compounds: an update. *Prostaglandins Leukot Essent Fatty Acids* 47: 1–12, 1992.
12. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, and Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med* 5: 698–701, 1999.
13. Gong P, Hu B, Stewart D, Ellerbe M, Figueroa YG, Blank V, Beckman BS, and Alam J. Cobalt induces heme oxygenase-1 expression by a hypoxia-inducible factor-independent mechanism in Chinese hamster ovary cells:regulation by Nrf2 and MafG transcription factors. *J Biol Chem* 276: 27018–27025, 2001.
14. He C, Gong P, Hu B, Stewart D, Choi ME, Choi AM, and Alam J. Identification of activating transcription factor 4 (atf4) as a nrf2 interacting protein: implication for heme oxygenase-1 gene regulation. *J Biol Chem* 276: 20858–20865, 2001.
15. Higashiyama K, Niiya K, Ozawa T, Hayakawa Y, Fujimaki M, and Sakuragawa N. Induction of c-fos protooncogene transcription and apoptosis by delta 12-prostaglandin J2 in human Pl-21 myeloid leukemia and RC-K8 pre-B lymphoma cells. *Prostaglandins* 52: 143–156, 1996.
16. Hiura TS, Kaszubowski MP, Li N, and Nel AE. Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. *J Immunol* 163: 582–591, 1999.
17. Igarashi K, Kataoka K, Itoh K, Hayashi N, Nishizawa M, and Yamamoto M. Regulation of transcription by dimerization of erythroid factor NF-E2 p45 with small Maf proteins. *Nature* 367: 568–572, 1994.
18. Inamdar NM, Ahn YI, and Alam J. The heme-responsive element of the mouse heme oxygenase-1 gene is an extended AP-1 binding site that resembles the recognition sequences for maf and NF-E2 transcription factors. *Biochem Biophys Res Commun* 221: 570–576, 1996.
19. Ishikawa T, Akimaru K, Nakanishi M, Tomokiyo K, Furuta K, Suzuki M, and Noyori R. Anti-cancer-prostaglandin-induced cell-cycle arrest and its modulation by an inhibitor of the ATP-dependent glutathione *S*-conjugate export pump (GS-X pump). *Biochem J* 336: 569–576, 1998.
20. Itoh K, Igarashi K, Hayashi N, Nishizawa M, and Yamamoto M. Cloning and characterization of a novel erythroid cell-derived CNC family transcription factor heterodimerizing with the small Maf family proteins. *Mol Cell Biol* 15: 4184–4193, 1995.
21. Itoh K, Ishii T, Wakabayashi N, and Yamamoto M. Regulatory mechanisms of cellular response to oxidative stress. *Free Radic Res* 31: 319–324, 1999.

22. Jiang C, Ting AT, and Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 391: 82–86, 1998.
23. Kasai K, Banba N, Hishinuma A, Matsumura M, Kakishita H, Matsumura M, Motohashi S, Sato N, and Hattori Y. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 facilitates thyroglobulin production by cultured human thyrocytes. *Am J Physiol Cell Physiol* 279: C1859-C1869, 2000.
24. Kersten S, Desvergne B, and Wahli W. Roles of PPARs in health and disease. *Nature* 405: 421–424, 2000.
25. Kitamura Y, Kakimura J-I, Matsuoka Y, Nomura Y, Gebicke-Haerter PJ, and Taniguchi T. Activators of peroxisome proliferator-activated receptor-γ (PPARγ) inhibit inducible nitric oxide synthase expression but increase heme oxygenase-1 expression in rat glial cells. *Neurosci Lett* 262: 129–132, 1999.
26. Koizumi T, Negishi M, and Ichigawa A. Induction of heme oxygenase by delta 12-prostaglandin J2 in porcine aortic endothelial cells. *Prostaglandins* 43: 121–131, 1992.
27. Koizumi T, Odani N, Okuyama T, Ichikawa A, and Negishi M. Identification of a cis-regulatory element for delta 12-prostaglandin J2-induced expression of the rat heme oxygenase gene. *J Biol Chem* 270: 21779–21784, 1995.
28. Kondo M, Oya-Ito T, Kumagai T, Osawa T, and Uchida K. Cyclopentenone prostaglandins as potential inducers of intracellular oxidative stress. *J Biol Chem* 276: 12076–12083, 2001.
29. Kotkow KJ and Orkin SH. Dependence of globin gene expression in mouse erythroleukemia cells on the NF-E2 heterodimer. *Mol Cell Biol* 15: 4640–4647, 1995.
30. Lee JH, Kim HS, Jeong SY, and Kim IK. Induction of p53 and apoptosis by delta 12-PGJ2 in human hepatocarcinoma SK-HEP-1 cells. *FEBS Lett* 368: 348–352, 1995.
31. Li N, Venkatesan MI, Miguel A, Kaplan R, Gujuluva S, Alam J, and Nel A Induction of heme oxygenase-1 expression in macrophages by diesel exhaust particle chemicals and quinones is mediated through antioxidant responsive elements. *J Immunol* 165: 3393–3401, 2000.
32. Maines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 37: 517–554, 1997.
33. Marini MG, Chan K, Casula L, Kan YW, Cao A, and Moi P. hMAF, a small human transcription factor that heterodimerizes specifically with Nrf1 and Nrf2. *J Biol Chem* 272: 16490–16497, 1997.
34. Moi P, Chan K, Asunis I, Cao A, and Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc Natl Acad Sci U S A* 91: 9926–9930, 1994.
35. Narumiya S and FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. *J Clin Invest* 108: 25–30, 2001.
36. Negishi M, Sugimoto Y, and Ichikawa A. Prostanoid receptors and their biological actions. *Prog Lipid Res* 32: 417–434, 1993.
37. Negishi M, Koizumi T, and Ichikawa A. Biological actions of delta 12-prostaglandin J2. *J Lipid Mediat Cell Signal* 12: 443–448, 1995.
38. Negishi M, Odani N, Koizumi T, Takahashi S, and Ichikawa A. Involvement of protein kinase in delta 12-prostaglandin J2-induced expression of rat heme oxygenase-1 gene. *FEBS Lett* 372: 279–282, 1995.
39. 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 GI block in cell cycle progression. *J Biol Chem* 263: 19764–19770, 1988.
40. Olive M, Krylov D, Echlin DR, Gardner K, Taparowsky E, and Vinson C. A dominant negative to activation protein-1 (AP-1) that abolishes DNA binding and inhibits oncogenesis. *J Biol Chem* 272: 18586–18594, 1997.
41. Otterbein LE, Bach FH, Alam J, Soares M, Lu HT, Wysk M, Davis RJ, Flavell RA, and Choi AMK. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med* 6: 422–428, 2000.
42. Poss KD and Tonegawa S. Heme oxygenase 1 is required for mammalian iron reutilization. *Proc Natl Acad Sci USA* 94: 10919–10924, 1997.
43. Poss KD and Tonegawa S. Reduced stress defense in heme oxygenase 1-deficient cells. *Proc Natl Acad Sci U S A* 94: 10925–10930, 1997.
44. Ramos-Gomez M, Kwak M-K, Dolan PM, Itoh K, Yamamoto M, Talalay P, and Kensler TW. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in *nrf2* transcripiton factor-deficient mice. *Proc Natl Acad Sci U S A* 98: 3410–3415, 2001.
45. Ricote M, Li AC, Wilson TM, Kelly CJ, and Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 391: 79–82, 1998.
46. Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, and Santoro MG. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. *Nature* 403: 103–108, 2000.
47. 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 U S A* 86: 8407–8411, 1989.
48. Smith WL. Prostanoid biosynthesis and mechanisms of action. *Am J Physiol* 263: F181-F191, 1992.
49. Willis D, Moore AR, Frederick R, and Willoughby DA. Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med* 2: 87–90, 1996.
50. Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, Ohta K, Kasahara Y, and Koizumi S. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. *J Clin Invest* 103: 129–135, 1999.

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1. A M Bataille, J E Manautou. 2012. Nrf2: A Potential Target for New Therapeutics in Liver Disease. *Clinical Pharmacology & Therapeutics* **92**:3, 340-348. [CrossRef]
2. Claudia N. Paiva, Daniel F. Feijó, Fabianno F. Dutra, Vitor C. Carneiro, Guilherme B. Freitas, Letícia S. Alves, Jacilene Mesquita, Guilherme B. Fortes, Rodrigo T. Figueiredo, Heitor S.P. Souza, Marcelo R. Fantappié, Joseli Lannes-Vieira, Marcelo T. Bozza. 2012. Oxidative stress fuels Trypanosoma cruzi infection in mice. *Journal of Clinical Investigation* . [CrossRef]
3. Young-Joon Surh, Hye-Kyung Na, Jong-Min Park, Ha-Na Lee, Wonki Kim, In-Soo Yoon, Dae-Duk Kim. 2011. 15-Deoxy-#12,14-prostaglandin J2, an electrophilic lipid mediator of anti-inflammatory and pro-resolving signaling. *Biochemical Pharmacology* **82**:10, 1335-1351. [CrossRef]
4. Ehsan Soleymaninejadian, Krishna Pramanik, Esmaeil Samadian. 2011. Immunomodulatory Properties of Mesenchymal Stem Cells: Cytokines and Factors. *American Journal of Reproductive Immunology* no-no. [CrossRef]
5. Min Li, Xiangjian Zhang, Lili Cui, Rui Yang, Lina Wang, Lingling Liu, Wei Du. 2011. The Neuroprotection of Oxymatrine in Cerebral Ischemia/Reperfusion Is Related to Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2)-Mediated Antioxidant Response: Role of Nrf2 and Hemeoxygenase-1 Expression. *Biological & Pharmaceutical Bulletin* **34**:5, 595-601. [CrossRef]
6. John D. Hayes , Michael McMahon , Sudhir Chowdhry , Albena T. Dinkova-Kostova . 2010. Cancer Chemoprevention Mechanisms Mediated Through the Keap1–Nrf2 Pathway. *Antioxidants & Redox Signaling* **13**:11, 1713-1748. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Ken Itoh , Junsei Mimura , Masayuki Yamamoto . 2010. Discovery of the Negative Regulator of Nrf2, Keap1: A Historical Overview. *Antioxidants & Redox Signaling* **13**:11, 1665-1678. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
8. Zahoor A Shah, Rung-chi Li, Abdullah S Ahmad, Thomas W Kensler, Masayuki Yamamoto, Shyam Biswal, Sylvain Doré. 2010. The flavanol (-)-epicatechin prevents stroke damage through the Nrf2/HO1 pathway. *Journal of Cerebral Blood Flow & Metabolism* **30**:12, 1951-1961. [CrossRef]
9. Iris F.F. Benzie , Sissi Wachtel-Galor . 2010. Vegetarian Diets and Public Health: Biomarker and Redox Connections. *Antioxidants & Redox Signaling* **13**:10, 1575-1591. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
10. Agnieszka Loboda, Alicja Jozkowicz, Jozef Dulak. 2010. HIF-1 and HIF-2 transcription factors — Similar but not identical. *Molecules and Cells* **29**:5, 435-442. [CrossRef]
11. Emilia Kansanen, Annukka M. Kivelä, Anna-Liisa Levonen. 2009. Regulation of Nrf2-dependent gene expression by 15-deoxy-#12,14-prostaglandin J2. *Free Radical Biology and Medicine* **47**:9, 1310-1317. [CrossRef]
12. Marcienne M. Wright, Junghyun Kim, Thomas D. Hock, Norbert Leitinger, Bruce A. Freeman, Anupam Agarwal. 2009. Human haem oxygenase-1 induction by nitro-linoleic acid is mediated by cAMP, AP-1 and E-box response element interactions. *Biochemical Journal* **422**:2, 353-361. [CrossRef]
13. Agnieszka Loboda , Anna Stachurska , Urszula Florczyk , Dominika Rudnicka , Agnieszka Jazwa , Joanna Wegrzyn , Magdalena Kozakowska , Krystyna Stalinska , Lorenz Poellinger , Anna–Liisa Levonen , Seppo Yla–Herttuala , Alicja Jozkowicz , Jozef Dulak . 2009. HIF-1 Induction Attenuates Nrf2-Dependent IL-8 Expression in Human Endothelial Cells. *Antioxidants & Redox Signaling* **11**:7, 1501-1517. [Abstract] [Full Text PDF] [Full Text PDF with Links]
14. Christopher M. Mahaffey, Hongqiao Zhang, Alessandra Rinna, William Holland, Philip C. Mack, Henry Jay Forman. 2009. Multidrug-resistant protein-3 gene regulation by the transcription factor Nrf2 in human bronchial epithelial and non-small-cell lung carcinoma. *Free Radical Biology and Medicine* **46**:12, 1650-1657. [CrossRef]

15. Andrea M. Vincent , Koichi Kato , Lisa L. McLean , Mary E. Soules , Eva L. Feldman . 2009. Sensory Neurons and Schwann Cells Respond to Oxidative Stress by Increasing Antioxidant Defense Mechanisms. *Antioxidants & Redox Signaling* **11**:3, 425-438. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
16. J CHANG, P TSAI. 2008. Prevention of methylmercury-induced mitochondrial depolarization, glutathione depletion and cell death by 15-deoxy-delta-12,14-prostaglandin J2. *NeuroToxicology* **29**:6, 1054-1061. [CrossRef]
17. Agnieszka Loboda , Agnieszka Jazwa , Anna Grochot-Przeczek , Andrzej J. Rutkowski , Jaroslaw Cisowski , Anupam Agarwal , Alicja Jozkowicz , Jozef Dulak . 2008. Heme Oxygenase-1 and the Vascular Bed: From Molecular Mechanisms to Therapeutic Opportunities. *Antioxidants & Redox Signaling* **10**:10, 1767-1812. [Abstract] [Full Text PDF] [Full Text PDF with Links]
18. E. Olatunde Farombi, Sangeeta Shrotriya, Hye-Kyung Na, Sung-Hoon Kim, Young-Joon Surh. 2008. Curcumin attenuates dimethylnitrosamine-induced liver injury in rats through Nrf2-mediated induction of heme oxygenase-1. *Food and Chemical Toxicology* **46**:4, 1279-1287. [CrossRef]
19. Xin Yu, Xiao-Guang Shao, Hong Sun, Yong-Nan Li, Jun Yang, Yan-Chun Deng, Yuan-Gui Huang. 2008. Activation of cerebral peroxisome proliferator-activated receptors gamma exerts neuroprotection by inhibiting oxidative stress following pilocarpine-induced status epilepticus. *Brain Research* **1200**, 146-158. [CrossRef]
20. K CHANG. 2008. Modulation of the expression of the # class of glutathione S-transferase by Andrographis paniculata extracts and andrographolide. *Food and Chemical Toxicology* **46**:3, 1079-1088. [CrossRef]
21. Shigeki Shibahara , Feng Han , Bin Li , Kazuhisa Takeda . 2007. Hypoxia and Heme Oxygenases: Oxygen Sensing and Regulation of Expression. *Antioxidants & Redox Signaling* **9**:12, 2209-2226. [Abstract] [Full Text PDF] [Full Text PDF with Links]
22. Hyun-Joung Lim, Kuy-Sook Lee, Seahyoung Lee, Jin-Hee Park, Hye-Eun Choi, Sang Hee Go, Hyun-Jeong Kwak, Hyun-Young Park. 2007. 15d-PGJ2 stimulates HO-1 expression through p38 MAP kinase and Nrf-2 pathway in rat vascular smooth muscle cells. *Toxicology and Applied Pharmacology* **223**:1, 20-27. [CrossRef]
23. Kazuhisa Takeda, Na-Ho Takahashi, Shigeki Shibahara. 2007. Neuroendocrine Functions of Melanocytes: Beyond the Skin-Deep Melanin Maker. *The Tohoku Journal of Experimental Medicine* **211**:3, 201-221. [CrossRef]
24. Hongqiao Zhang, Honglei Liu, Dale A. Dickinson, Rui-Ming Liu, Edward M. Postlethwait, Yannick Laperche, Henry Jay Forman. 2006. #-Glutamyl transpeptidase is induced by 4-hydroxynonenal via EpRE/Nrf2 signaling in rat epithelial type II cells. *Free Radical Biology and Medicine* **40**:8, 1281-1292. [CrossRef]
25. Marta P Pereira, Olivia Hurtado, Antonio Cárdenas, David Alonso-Escolano, Lisardo Boscá, José Vivancos, Florentino Nombela, Juan C Leza, Pedro Lorenzo, Ignacio Lizasoain, María A Moro. 2005. The Nonthiazolidinedione PPAR# Agonist L-796,449 Is Neuroprotective in Experimental Stroke. *Journal of Neuropathology and Experimental Neurology* **64**:9, 797-805. [CrossRef]
26. Aida Abate, Guang Yang, Ronald J. Wong, Henning Schroder, David K. Stevenson, Phyllis A. Dennery. 2005. Apigenin decreases hemin-mediated heme oxygenase-1 induction. *Free Radical Biology and Medicine* **39**:6, 711-718. [CrossRef]
27. Ken Itoh, Masayuki Yamamoto. 2005. Regulatory Role of the COX-2 Pathway in the Nrf2-Mediated Anti-Inflammatory Response. *Journal of Clinical Biochemistry and Nutrition* **37**:1, 9-18. [CrossRef]
28. EUN-HEE KIM, DO-HEE KIM, HYE-KYUNG NA, YOUNG-JOON SURH. 2004. Effects of Cyclopentenone Prostaglandins on the Expression of Heme Oxygenase-1 in MCF-7 Cells. *Annals of the New York Academy of Sciences* **1030**:1, 493-500. [CrossRef]
29. Tae Yamamoto, Keigyou Yoh, Akira Kobayashi, Yukio Ishii, Shigeo Kure, Akio Koyama, Tohru Sakamoto, Kiyohisa Sekizawa, Hozumi Motohashi, Masayuki Yamamoto. 2004. Identification of

polymorphisms in the promoter region of the human NRF2 gene. *Biochemical and Biophysical Research Communications* **321**:1, 72-79. [CrossRef]

30. J Liu. 2004. Thiol antioxidant and thiol-reducing agents attenuate 15-deoxy-#12,14-prostaglandin J2-induced heme oxygenase-1 expression. *Life Sciences* **74**:19, 2451-2463. [CrossRef]
31. Pengfei Gong, Arthur I Cederbaum, Natalia Nieto. 2004. Heme oxygenase-1 protects HepG2 cells against cytochrome P450 2E1-dependent toxicity. *Free Radical Biology and Medicine* **36**:3, 307-318. [CrossRef]
32. Alicja Józkowicz, Anneliese Nigisch, Joanna We#grzyn, Guenter Weigel, Ihor Huk, Józef Dulak. 2004. Opposite effects of prostaglandin-J2 on VEGF in normoxia and hypoxia: role of HIF-1. *Biochemical and Biophysical Research Communications* **314**:1, 31-38. [CrossRef]
33. I Jowsey. 2003. Expression of the murine glutathione S-transferase #3 (GSTA3) subunit is markedly induced during adipocyte differentiation: activation of the GSTA3 gene promoter by the pro-adipogenic eicosanoid 15-deoxy-#12,14-prostaglandin J2. *Biochemical and Biophysical Research Communications* **312**:4, 1226-1235. [CrossRef]
34. 2003. Trend of Most Cited Papers (2001-2002) in ARS. *Antioxidants & Redox Signaling* **5**:6, 813-815. [Citation] [Full Text PDF] [Full Text PDF with Links]
35. Joanne E. Natale , Farid Ahmed , Ibolja Cernak , Bogdan Stoica , Alan I. Faden . 2003. Gene Expression Profile Changes Are Commonly Modulated across Models and Species after Traumatic Brain Injury. *Journal of Neurotrauma* **20**:10, 907-927. [Abstract] [Full Text PDF] [Full Text PDF with Links]
36. Kazuhiro Nakaso, Hidetaka Yano, Yoko Fukuhara, Takao Takeshima, Kenji Wada-Isoe, Kenji Nakashima. 2003. PI3K is a key molecule in the Nrf2-mediated regulation of antioxidative proteins by hemin in human neuroblastoma cells. *FEBS Letters* **546**:2-3, 181-184. [CrossRef]
37. Ian R. Jowsey, Paul R. Murdock, Gary B. T. Moore, Gregory J. Murphy, Stephen A. Smith, John D. Hayes. 2003. Prostaglandin D2 synthase enzymes and PPAR# are co-expressed in mouse 3T3-L1 adipocytes and human tissues. *Prostaglandins & Other Lipid Mediators* **70**:3-4, 267-284. [CrossRef]
38. Pengfei Gong, Daniel Stewart, Bin Hu, Charles Vinson, Jawed Alam. 2002. Multiple basic-leucine zipper proteins regulate induction of the mouse heme oxygenase-1 gene by arsenite. *Archives of Biochemistry and Biophysics* **405**:2, 265-274. [CrossRef]