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Prostaglandin E2 Potentiates Heat Shock-Induced Heat Shock Protein 72 Expression in A549 Cells

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Abstract

The heat shock (HS) response is an important cytoprotective response comprising expression of heat shock proteins (HSP) and orchestrated by the heat/stress-induced transcription factor, heat shock factor-1 (HSF-1). Previous studies suggest that the activation threshold and magnitude of the HS response may be modified by treatment with arachidonic acid (AA). We analyzed the effect of exogenous AA and its metabolites, PGE₂, LTD₄, and 15-HETE on HSF-1-dependent gene expression in A549 human respiratory epithelial-like cells. When added at 1 μM, PGE₂ much more than LTD₄, but not 15-HETE increased activity of a synthetic HSF-1-dependent reporter after HS exposure (42°C for 2h), but had no effect in the absence of HS. Exposing A549 cells to HS stimulated release of PGE₂ and treatment with the cyclooxygenase inhibitor, ibuprofen, reduced HS-induced HSF-1-dependent transcription. PGE₂ increased HS-induced HSP72 mRNA and protein expression but EMSA and Western blot analysis failed to show an effect on HSF-1 DNA binding activity or post-translational modification. In summary, we showed that HS stimulates generation of PGE₂, which augments generation of HSPs. The clinical consequences of this pathway have yet to be determined.

Keywords

Heat shock; HSP-72; Prostaglandin E2; Arachidonic Acid

Introduction

The heat shock (HS) response is an evolutionary conserved response to stress, including high temperatures, which is characterized by the generation of a family of heat shock

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proteins (HSPs), including HSP72 (HSPa1a, b) 1. In vertebrates, HSP genes are regulated by the heat/stress activated transcription factor, heat shock factor-1 (HSF-1) 2, which is required for HS-induced HSP expression 3. When exposed to heat or other stresses such as ischemia and aging, HSF-1 undergoes a stepwise activation including trimerization, nuclear translocation, and independent acquisition of DNA binding and transactivating capacities. While HSF-1 trimerization is sufficient for it to bind to heat shock response elements (HSE) in the promoter regions of HSPs and other genes, transcriptional activation requires further modifications, including phosphorylation and sumoylation, that are detectable as a decrease in electrophoretic mobility on SDS-PAGE 4, 5.

Our group has shown that the activation of HS gene expression is both temperature- and time-dependent 6. We found that exposing A549 human lung epithelial-like cells to an incubation temperature as low as 38.5°C was sufficient to activate HSP72 expression, the magnitude of which increased progressively as incubation temperatures were incrementally increased from 38.5°C to 41°C. In contrast to the proportional response of HSP72 gene expression to increasing temperature, the DNA-binding activity of HSF-1 was activated to similar levels in cells exposed to 38.5°, 39.5°, and 41°C. However, the electrophoretic mobility of HSF-1 on Western blots progressively decreased with increasing incubation temperature between 38.5°C and 41°C and the activity of an HSF-1-dependent reporter plasmid increased, suggesting that the extent of HSF-1 post-translational modification and its trans-activating potential are proportional to temperature in this range.

These results demonstrate that the thermal threshold for activation of the HS response is variable and the magnitude of HSP gene expression is proportional to the magnitude of stress. Previous studies suggest that the thermal threshold for activation of the HS response and the magnitude of HSP expression may also be modified by exposure to certain inflammatory mediators, including arachidonic acid (AA) 5, 7 and type I interferons 5, 8. Jurivich *et al.* 5, 7 showed that treating HeLa cells with 10 µM AA prior to a 30 min thermal stress reduced the threshold for HSP gene expression from 41°C to 39°C. Treatment with higher levels of AA (20µM) was sufficient to activate HSP expression at 37°C in these cells. The capacity of immunologic mediators such as AA to reduce the threshold for activation of the HS response may explain the lower thermal threshold required to activate the HS response *in vivo* especially during inflammation 9, 10

Free AA is formed by release from the *sn*-2 position of phospholipids by A₂ phospholipases and is the precursor for the generation of important lipid second messengers including prostaglandins (PGs), leukotrienes (LTs), and hydroxyeicosatetraenoic acids (HETEs) 5, 11. Cytoplasmic phospholipase A₂-α (cPLA₂-α)-mediated AA release is the rate-limiting step in the generation of downstream mediators in most systems 5, 12 and is responsible for the stimulated release of AA *in vivo*. cPLA₂-α knockout mice lack the ability to generate LTs, PGs, or PAF in a variety of cell types utilizing a wide range of stimuli 13.

The fate of AA once released depends on the presence and activity of downstream enzymes responsible for the formation of specific lipid mediators. The three best-described pathways for processing AA are cyclooxygenase metabolism resulting in PG formation, 5-lipoxygenase activity forming LTs, and 15-lipoxygenase reactions yielding 15-HETEs 5, 11. Of these families of AA metabolites, only 15-deoxy-Delta 12,14-prostaglandin J2, the ultimate metabolite of cyclooxygenase-derived PGD₂, has been shown capable of activating HSF-1 and HSP gene expression 14.

The purpose of our study was to determine whether other AA metabolites activate or modify the HS response. We analyzed the effect of exogenous AA metabolites on HSF-1-dependent reporter plasmid expression, HSF-1 activation, and HSP72 mRNA and protein generation in

the human A549 respiratory epithelial-like adenocarcinoma cell line. PGE₂ to a much greater extent than LTD₄, but not 15-HETE, increased HSF-1-dependent gene expression.

Materials and Methods

Cell culture and Heating protocol

The human alveolar type II epithelial-like cell line, A549 cells (American Type Cell Collection; Manassas, VA), were maintained in RPMI 1640 containing 10% defined fetal bovine serum (FBS; Hyclone, Logan, UT) at 37°C in 5% CO₂-enriched air. Cells were adjusted to 1×10^5 cells/ml and plated in a 24-well plate and incubated at 37°C for 24 h, prior to exposure to HS (42°C for 2 h). Some cells were exposed to PGE₂, 15-HETE, AA or LTD₄ added simultaneously with HS. In other experiments, cells were pretreated with 5 μM ibuprofen added 30 min prior to HS. Stock solutions of 0.1 mg/ml 15-HETE and LTD₄ and 100 mg/ml AA in ethanol were purchased from Cayman Chemical (Ann Arbor, MI). PGE₂ was purchased from Cayman Chemical and dissolved in ethanol to produce a 10 mg/ml stock solution. Stock solutions were diluted further in culture medium to achieve the final concentrations reported. To control for the effects of ethanol, control cells were treated with 0.4% ethanol. Ibuprofen sodium was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in PBS to produce a 10 mM stock solution.

Western blot

A549 cells were incubated with or without AA or one of its metabolites and with or without a 2 h HS at 42°C and 4 h recovery at 37°C for a total of 6 h. Following treatment, cells were lysed in RIPA buffer (Teknova; Hollister, CA) containing protease inhibitors (Roche, Palo Alto, CA) and phosphatase inhibitors (Sigma, St. Louis, MO), resolved on 10% SDS-polyacrylamide gels, and transferred to PVDF membrane (Millipore, Billerica, MA). The membranes were blocked for 1 h at room temperature in blocking buffer (TBS-T (10 mM Tris-HCl, pH7.5, 136 mM NaCl, 2 mM KCl, 0.1% Tween 20) containing 5% nonfat dry milk). Following blocking, the membranes were washed with TBS-T, and incubated with rabbit antibodies against HSP72 (Stressgen; Ann Arbor, MI), β-tubulin (Millipore), or rat anti-HSF-1 (Santa Cruz; Santa Cruz, CA) in blocking buffer for 2 h. After primary antibody reactions, the membranes were washed with TBS-T, incubated for 1 h with horseradish peroxidase-conjugated secondary antibody (Santa Cruz) and developed with a chemiluminescence detection system (Renaissance™; New England Nuclear; Boston, MA), quantified by direct imaging (Fuji gel documentation system and ImageGauge software).

HSE reporter analysis

For stable transfections, A549 cells were co-transfected with a luciferase reporter construct driven by a synthetic promoter composed of multiple HSEs and the TATA-like promoter region from the Herpes simplex virus thymidine kinase gene (pHSE-Luc) along with the blasticidin resistance plasmid, pcDNA6/TR, using Fugene-6 transfection agent. Transfectants were selected using 4 μg/ml blasticidin, and maintained in media containing 2 μg/ml blasticidin and cloned by limiting dilution. Single colonies were isolated, analyzed for HS-induced activation of the HSE-containing promoter and a single clone was used for further studies. For reporter assays, 1×10^5 cells/ml were plated in blasticidin-free medium for 24 h, then were incubated with or without AA or one of its metabolites and with or without a 2 h HS and 4 h recovery at 37°C for a total of 6 h. Controls were treated with the AA/PGE₂ vehicle, EtOH at 1%, the highest concentration present in the AA/PGE₂ dose response. The cells were then lysed in 1× Cell culture lysis buffer (Promega; Madison, WI) and luciferase activity was analyzed using a commercial luciferase assay kit (Promega).

Quantitative Reverse Transcription/Polymerase Chain Reaction Assay (qRT-PCR)

Total RNA from 2×10^6 A549 cells was isolated using RNeasy (Qiagen; Valencia, CA) and contaminating DNA was eliminated using DNase I digestion (Qiagen). RNA was reverse-transcribed using 1 μ g total RNA and oligo-dT primer using a cDNA synthesis kit according to the manufacturer's protocol (Promega). Duplicate 25 μ l real-time PCR reactions were performed in 96 well plates using SYBR-Green reaction mix (Biorad; Hercules, CA) and Biorad iCycler according to the supplier's protocol with the following forward and reverse primers: glyceraldehydes-3 phosphate dehydrogenase (GAPDH), 5'-agcctcgtcccctagacaaaat and 5'-tggcaacaatctccactttgc; and HSP72, 5'-accaagcagacgcagatcttc and 5'-agcctcaagatcatcagcaatg. Data were quantified using the Gene Expression Ct Difference method described by Schefe *et al.*¹⁵ and standardized to levels of the housekeeping gene, GAPDH, using Ct values automatically determined by the thermocycler. Briefly, the efficiency of amplification for each primer pair was calculated using the equation:

$$\text{Efficiency} = 10^{(1/\text{slope})} - 1$$

where slope is measured from the linear portion of the log fluorescence vs. cycle number curve. The expression of the gene of interest (GOI) in each sample relative to a reference, untreated 37°C cells, was calculated using the equation:

$$\text{Relative GOI expression} = \frac{(1 + \text{efficiency}_{GOI})^{-\Delta C_t(GOI)}}{(1 + \text{efficiency}_{GAPDH})^{-\Delta C_t(GAPDH)}}$$

where ΔC_t (GOI) and ΔC_t (GAPDH) is the difference in threshold cycle between sample and reference for the gene of interest and GAPDH, respectively.

Prostaglandin E Assay

Media from cell cultures was assayed in triplicate for PGE₂ using an EIA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's protocol.

Electrophoretic Mobility Shift Assays (EMSA)

Nuclear extracts from A549 cells were prepared according to the method of Schreiber *et al.*¹⁶ as described earlier¹⁷ and total protein concentration was measured using a commercial reagent (Biorad) against a bovine serum albumin standard curve. Double-stranded oligonucleotide containing the HSE corresponding to -107/-83 of the human Hspa1a promoter¹⁸ (5'-GATCTCGGCTGGAATATTCCTGACCTGGCAGCCGA-3') was generated by annealing complementary strands and radiolabeling using T4 polynucleotide kinase (Promega) and [γ -³²P]ATP according to the manufacturer's protocol. The DNA-protein complexes were then electrophoretically resolved on 4% nondenaturing polyacrylamide gels. The dried gels were analyzed by phosphorimaging (Molecular Dynamics, Sunnyvale, CA) and exposed to X-ray film. Where indicated, 30-fold excess unlabeled competitor (Hspa1a promoter) or nonspecific oligonucleotide or 1:20 dilution of anti-HSF-1 antibody (Santa Cruz) were incubated with the nuclear extracts for 30 min at room temperature before the addition of the radiolabeled probe.

Statistical Analysis

Data are presented as mean \pm SE. Differences among groups were analyzed by applying a Tukey Honestly Significant Difference test to a one-way ANOVA. Differences with $p < 0.05$ were considered significant.

Results

Effect of AA and metabolites on HSF-1-dependent gene expression

Adding exogenous AA has been reported to augment activation of HSF-1-dependent gene expression. Since AA is predominantly metabolized to three families of potent biochemical mediators, we performed an initial experiment to identify the AA metabolite family that augments HSF-1-dependent gene expression using A549 cells stably expressing a HSF-1-responsive promoter as we have previously described⁶ (Fig. 1A). Cells were treated with 1 μ M concentrations of AA, 15-HETE, LTD₄, or PGE₂. Controls were incubated in medium alone or with 0.4% ethanol, the diluent used for AA and its metabolites. Treated cells were either exposed to HS for 2 h at 42°C followed by 4 h recovery at 37°C or were incubated for 6 h at 37°C without HS, and luciferase levels were measured. Ethanol-treated controls exhibited luciferase activity virtually identical with untreated cells. PGE₂ and LTD₄ augmented HS-induced luciferase expression compared with ethanol-treated controls; however, the effect was much greater with PGE₂. Neither PGE₂ nor any of the other lipid mediators tested activated luciferase expression in the absence of HS. Analysis of the PGE₂ dose response indicated that PGE₂ concentrations below 1 μ M are not sufficient to modify HSF-1-dependent transcription (Fig. 1B). Because Jurivich *et al.*⁷ demonstrated that 20 μ M AA was sufficient to activate HSP72 expression at 37°C and 10 μ M AA synergized with heat to activate HSP72 transcription in HeLa cells, we analyzed the effect of AA concentrations between 1 and 20 μ M on activation of an HSF-1-responsive reporter plasmid (1C). AA at concentrations of 10 and 20 μ M tended to increase HS-induced reporter plasmid activation, but the difference did not reach statistical significance. AA at concentrations up to 20 μ M had no effect on reporter activity in the absence of HS. Since AA is a precursor for PGE₂, we analyzed the effect of HS on PGE₂ synthesis without and with exogenous AA (1D).

While HS did not induce a detectable increase from basal PGE₂ levels in ethanol-treated control cells, combined treatment with 20 μ M AA and exposure to 42°C increased PGE₂ levels by 6.6-fold compared with ethanol-treated cells incubated at 37°C and by 2.8-fold vs. cells treated with AA-treated cells at 37°C. Finally, to determine whether generation of endogenous PGE₂ contributed to HS-induced activation of HSF-1-dependent gene expression, we analyzed the effect of pretreatment with the cyclooxygenase inhibitor, ibuprofen, on HS-induced reporter plasmid activity (1E). Ibuprofen (5 μ M) added 30 min prior to HS reduced the HS-induced reporter plasmid activity by 44% compared with heat-shocked cells incubated without ibuprofen, but had no effect on activity in 37°C cells.

PGE₂ Enhances HS-induced HSP72 expression

To determine whether PGE₂ has similar effects on HS-induced expression of endogenous HSF-1-responsive genes, we analyzed the effect of adding 1 μ M PGE₂ with or without HS on levels of HSP72 mRNA and protein. Non-transfected A549 cells were incubated with 1 μ M PGE₂ or 0.4% ethanol with or without 2 h HS at 42°C followed by recovery at 37°C. HSP72 mRNA levels were analyzed by quantitative RT-PCR after 2 h (Fig. 2A) and HSP72 protein levels were analyzed by Western blotting after the 2 h HS and 4 h recovery (Fig. 2B, C). PGE₂ exerted effects on HSP72 gene expression that were similar to its effects on activation of the HSF-1-responsive reporter plasmid. PGE₂ increased HS-induced HSP72 mRNA levels by 42% and HSP72 protein levels by 77%, but in the absence of HS, PGE₂ had no detectable effect on HSP72 expression.

Effect of PGE₂ on HSF-1 activation

HSF-1 exists as a monomer and homotrimerizes in response to stress^{5, 19}. Mutational analyses of HSF-1 suggests that homotrimerization is sufficient for HSF-1 to attain capacity for specific, high affinity DNA binding to three contiguous inverted nGAAn dyad repeats

20–22. However, the additional gain of transcriptional activating activity requires further posttranslational modification of the HSF-1 trimers, including phosphorylation^{23–26}, SUMOylation²⁷ and possibly oxidation^{5, 28}. To determine whether PGE₂ exerted its effects on HSF-1 trimerization/DNA binding or covalent modification, we analyzed nuclear extracts from A549 cells incubated with 1 μM PGE₂ or 0.4% ethanol with or without 1 or 2 h HS at 42°C for HSF-1 DNA binding activity by EMSA (Fig. 3A) and covalent modification by analyzing the electrophoretic mobility of HSF-1 on an SDS-PAGE/Western blot (Fig. 3C). As expected, exposure to HS increased both DNA-binding activity and apparent molecular weight of HSF-1, but PGE₂ did not cause further increases in these measures of HSF-1.

Discussion

Jurivich *et al.* showed that treatment with exogenous AA synergizes with heat to activate HSF-1 and induce HSP gene expression, but only at high AA concentrations^{5, 7, 29–31}. In our current study, we extend these observations by demonstrating that the AA metabolites PGE₂ to a much greater extent than LTD₄ but not 15-HETE, augmented HS-induced HSF-1-dependent gene expression.

Transcription of heat shock protein genes is predominantly regulated by the heat-activated transcription factor, HSF-1, but other transcriptional regulators, including STAT-1, NF-IL-6 and TFIID, have also been shown to bind to HSF-1 with variable effects on HSF-1 function. For example, STAT-1 and HSF-1 synergistically activated transcription of HSP72 and HSP90^{5, 32}, whereas HSF-1 and NF-IL-6 are mutually antagonistic^{33, 34}. Similarly, Yuan and Gurley³⁵ reported that HSF-1 could bind both TATA-binding protein (TBP) and the TFIIB transcription factor complex, and implied that these interactions determine whether the complex is dysfunctional or a transcriptionally competent pre-initiation complex. We initially analyzed the effect of AA and its metabolites on activity of a luciferase reporter driven by an artificial HSF-1-responsive promoter stably transfected into A549 cells. The promoter, which contains multiple HSEs and the TATA-like promoter region from the Herpes simplex virus thymidine kinase gene, exhibited an 84-fold increase in activity following exposure to HS. Utilizing this system, we showed that HS-induced transcriptional activation was further enhanced by 1 μM PGE₂. Since this promoter lacks binding sequences for transcriptional activators other than HSF-1, our results suggest that PGE₂ and LTD₄ increase HSF-1-dependent transcription. Jurivich *et al.*^{5, 7} demonstrated in HeLa cells that 10 μM AA reduced the thermal threshold for heat-induced HSF-1 activation and HSP72 transcription and 20 μM AA was sufficient to induce sub-maximal activation of HSF-1 and HSP72 transcription at 37°C. Our results suggested a similar interaction between AA and heat in activating HSF-1-dependent gene expression, but the trend in increased HS-induced HSF-1-responsive reporter plasmid activity in cells treated with 10 and 20 μM AA did not reach statistical significance. The AA concentrations analyzed in these studies appears to be achievable *in vivo*, especially in the presence of inflammation. For example, concentrations of AA have been reported to be ~0.5–1 μM in resting leukocytes³⁶, 15 μM in isolated islets of Langerhans³⁷, and 100 μM in the inflamed skin tissue of psoriasis³⁸. However, whether exogenous AA exerted its effects directly or through effects of one or more of its metabolites was not addressed in these studies.

In this study, we demonstrated that exposing A549 cells to HS tended to increase secretion of PGE₂, but the effect of HS on PGE₂ generation was much greater in the presence of exogenous AA, suggesting that HS may exert its effects predominantly distal to AA release from membrane lipids. Furthermore, we found that treating A549 cells with the cyclooxygenase inhibitor, ibuprofen, attenuated the HS-induced activation of HSF-1-dependent gene expression. Taken with our demonstration that exogenous PGE₂ increases HS-induced activity of an HSF-1-responsive reporter plasmid, these results suggest that HS

induces generation of PGE₂, which is required for maximal HSF-1-dependent gene transcription.

Surprisingly, we found that PGE₂ levels ≤100 nM had no effect on HSF-1-dependent gene expression. Most studies show that PGE₂ at nanomolar levels activates a family of membrane-associated G-protein coupled receptors, EP1 through EP4, all of which are expressed in epithelial cell culture lines³⁹. Yano et al⁴⁰ showed in A549 cells that 10 nM PGE₂ increased proliferation and activation of extracellular signal-regulated kinase activation via EP3 and 4 engagement, raising questions about whether 1 μM PGE₂ modifies HSF-1-dependent gene expression through alternative mechanisms. The PGD₂ metabolite and potent HSF-1 activator, 15-deoxy-Delta^{12,14}-prostaglandin J₂, activates the nuclear transcription factor PPARγ^{41, 42}. Several studies show that non-lipid PPARγ agonists also activate HSF-1 and HSP gene expression. However, to our knowledge, there are no reports of PGE₂ exhibiting PPARγ agonist activity. Of note, Yamaki *et al.*⁴³ found that 100 μM but not 1 μM PGE₂ activated src protein tyrosine kinase in A549 cells; however, they did not identify the mechanisms through which PGE₂ stimulated this cell response.

Other groups have shown that cyclooxygenase metabolites, including PGE₂, enhance activation of the HS response in other cell systems and tissues while inhibition of cyclooxygenase reduces this activation. Togo *et al.*⁴⁴ showed that treating rats with PGE₁ enhanced HSP70 generation in residual liver and reduced acute liver failure after massive hepatectomy. Hwang *et al.*⁴⁵ reported that the cyclopentenone prostaglandin, 15-deoxy-Delta^(12,14)-prostaglandin J₂ (15d-PGJ₂), induced HSP70 expression in endothelial cells. Xu *et al.*⁴⁶ showed that PGA₁ pretreatment increased HSP70 and glucose-regulated-protein 78 (GRP78) levels and reduced brain infarct size after middle cerebral artery occlusion in rats. Warzecha *et al.*⁴⁷ demonstrated that the increase in pancreatic HSP70 levels induced by ischemic preconditioning in a cerulein-induced pancreatitis model was reduced by pretreatment with COX-2 inhibition. Matsuo *et al.*⁴⁸ demonstrated that treating rats with PGE₁ increased hepatic expression of several HSP genes after ischemia reperfusion. Ethridge *et al.*⁴⁹ found that overexpression of COX-2 reduced HSP70 mRNA and protein after HS in rat intestinal epithelial cells, suggesting that some COX-2 metabolites may reduce HSP expression in some cell types. In contrast with these studies largely demonstrating positive regulation of the HS response by prostaglandins, other investigators have shown that cyclooxygenase inhibitors, including salicylate^{29, 30} and indomethacin³¹, activated HSF-1 to its DNA binding form in HeLa cells. However these effects were only found when the drugs were used at toxic levels and neither drug activated expression of HSF-1-dependent genes. Housby *et al.*⁵⁰ found that the cyclooxygenase inhibitor, sulindac, activated HSF-1 DNA binding activity and HSP70B transcription; however, like the other studies, these effects were only seen at toxic levels of the drug. Collectively, these data suggest that endogenous PGE₂ generation may contribute to heat shock gene expression through activation of HSF-1 to a transcriptionally active form.

In summary, we have shown that exogenous PGE₂, added at levels achieved *in vivo*, increase the magnitude of HS-induced gene expression. The mechanism underlying this effect and its clinical consequences are not yet known.

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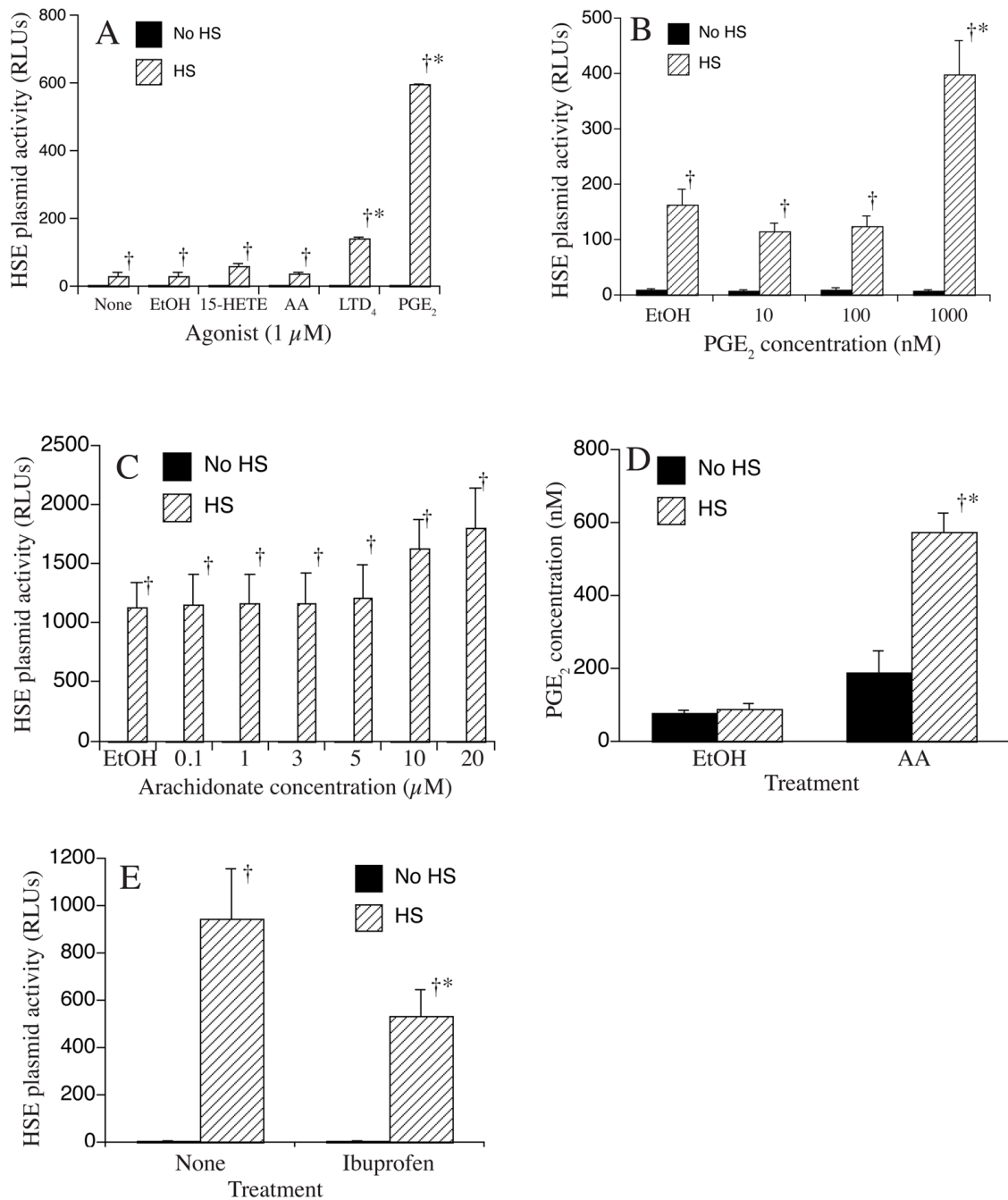


Figure 1. Effect of PGE₂ and other stimuli on HS-induced activation of a HS-responsive reporter construct

A549 cells stably transfected with a HS-responsive luciferase reporter plasmid were incubated for 6 h with or without a 2 h 42°C HS and coincident treatment with medium alone (none), vehicle (0.4% EtOH), 1 μ M of AA or indicated AA metabolite (A), the indicated concentration of PGE₂ (B), the indicated concentration of AA (C), 20 μ M AA (D) or 30 min pretreatment with 5 μ M ibuprofen (E). The cells were then lysed and luciferase measured (A-C, E) and expressed as fold-change compared with untreated controls without HS or PGE₂ measured by EIA (D). Mean \pm SE, n = 4 (A-C, E) or 3 (D); * and † denotes

$p < 0.05$ vs. untreated, HS-exposed controls and cells receiving the same treatment but without HS, respectively.

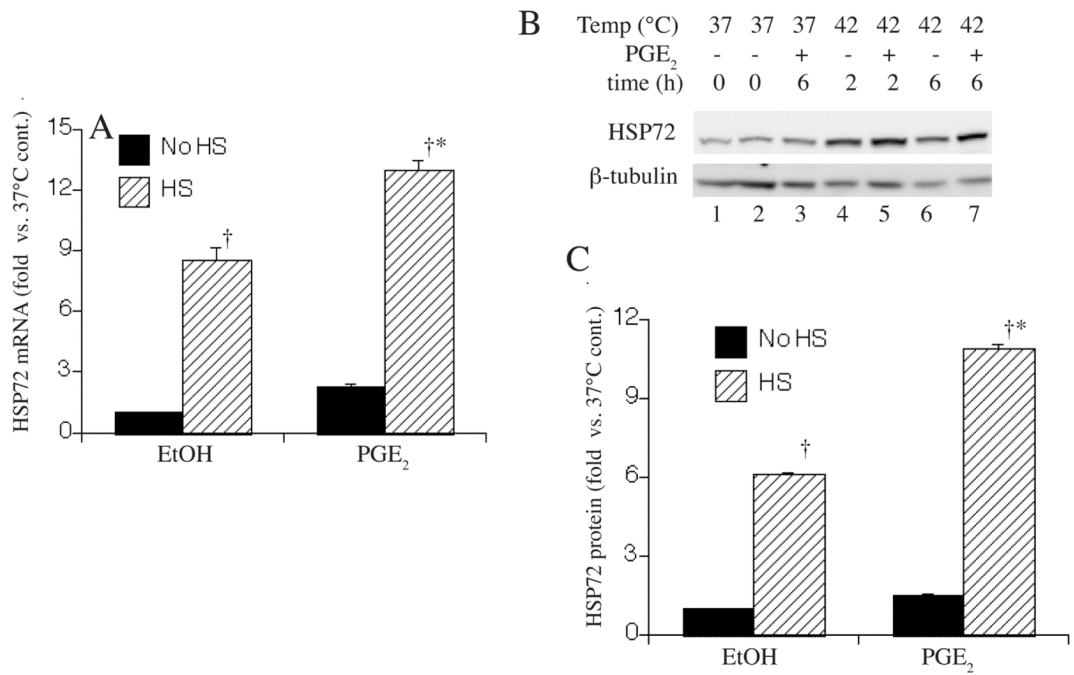
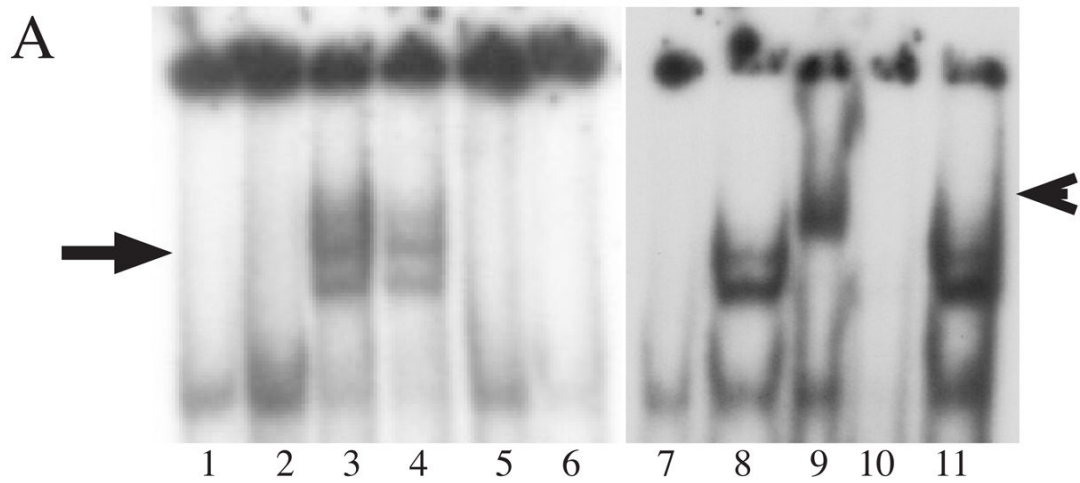


Figure 2. Effect of PGE₂ on HS-induced activation of HSP72 expression

A549 cells were incubated for 6 h with or without a 2 h, 42°C HS and with 1 μM PGE₂ or 0.4% ethanol and HSP72 mRNA was quantified by real-time RT-PCR standardized to GAPDH (A) and protein was quantified by Western blotting with β-tubulin as a control (representative of 4 similar gels in B). The bands were quantified by direct imaging of the chemiluminescent bands, expressed as a ratio to β-tubulin, and as fold change vs untreated cells without HS (C). Data are expressed as mean±SE, n = 4; * and † denotes p<0.05 vs. untreated, HS-exposed controls and cells receiving the same treatment but without HS, respectively.

Temp (°C)	37	37	42	42	42	42	37	42	42	42	42
PGE ₂	-	+	-	+	-	+			ss	cc	ns
time (h)	0	6	2	2	6	6	0	2	2	2	2



Temp (°C)	37	37	42	42	42	42	42	42
PGE ₂	-	+	-	+	-	+	-	+
time (h)		6	1	1	2	2	6	6

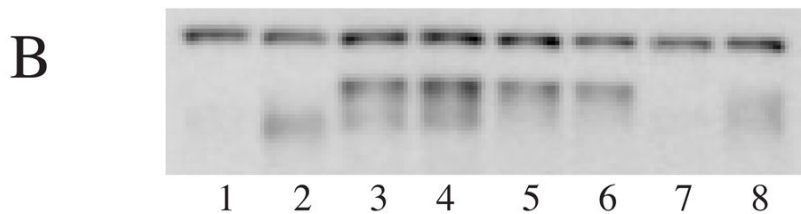


Figure 3. Effect of PGE₂ on HSF-1 activation

Nuclear extracts were collected from A549 cells treated with 1 μ M PGE₂ or 0.4% ethanol with or without HS at 42°C and analyzed for HSF-1 DNA binding activity by EMSA (representative of 4 similar autoradiographs in A; HSF-1-shifted complex indicated by arrow). HSF-1 binding was confirmed by supershifting with anti-HSF-1 antibody (ss, lane 9; indicated by arrowhead) and competition with 30X excess of unlabeled hspa1a oligonucleotide (cc, lane 10). Competition with a nonspecific (ns) oligonucleotide is shown as a negative control in lane 11. B. Covalent modification of HSF-1 was analyzed by measuring the electrophoretic mobility of HSF-1 in nuclear extracts by Western blotting; a representative of 3 similar blots is shown.