Tumour necrosis factor-alpha stimulates increased expression of prostaglandin endoperoxide H synthase Type 2 mRNA in amnion-derived WISH cells

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ABSTRACT

We have evaluated the mechanism by which tumour necrosis factor-alpha (TNF-α) induces increased prostaglandin (PG) biosynthesis in amnion-derived WISH cells. WISH cells were treated with 50 ng/ml TNF-α or vehicle for 0–24 h. PGE2 production was stimulated by TNF-α within 2 h and continued to accumulate for at least 24 h. Increased prostaglandin endoperoxide H synthase (PGHS)-2 mRNA expression was evident within 30 min and was highest by 1 h, returning to unstimulated levels by 2 h. The PGHS-2 mRNA was re-induced at 8 h and was also elevated at 16 h. Immunoreactive PGHS-2 protein was nearly undetectable in control cells. However, within 30 min of TNF-α treatment, PGHS-2 protein was elevated and was induced for at least 16 h suggesting rapid production of both the PGHS-2 mRNA and protein. Transcription run-on assays indicated that the initial increase in the PGHS-2 mRNA was due to a 20-fold increase in the rate of transcription. The PGHS-2 mRNA decayed with an apparent half-life of 1 h in TNF-α-stimulated WISH cells. Induction of PGHS-2 expression proceeded in the presence of 10 µg/ml cycloheximide which agrees with the classification of PGHS-2 as an immediate early gene. These results indicate that a bi-phasic induction of the PGHS-2 mRNA is due, in part, to an initial transcriptional activation which results in rapid and continued synthesis of the PGHS-2 protein. This may be a unique characteristic of amnion cells which may be partially responsible for increased PG concentrations in the amniotic fluid during infection-associated preterm labour.

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INTRODUCTION

The presence of an intrauterine infection induces the production of inflammatory cytokines which have been implicated in the pathology of infection-associated preterm labor (Dudley & Trautman 1994). In particular, the concentrations of tumour necrosis factor-alpha (TNF-α) are elevated in the amniotic fluid of patients with intrauterine infection and preterm labour (Hillier et al. 1993, Laham et al. 1994). Using a non-human primate model for chorioamnionitis, Gravett and colleagues (1994) determined that TNF-α is the first detectable inflammatory cytokine in the amniotic fluid after bacterial colonisation, preceding both interleukin (IL)-1 and IL-6. TNF-α is also capable of regulating the expression of IL-1 receptors in the human amnion (Bry et al. 1993). These observations suggest that the production of TNF-α early in the host response to infection might play an important role in the pathophysiology of preterm labour.

There are at least two forms of the enzyme that catalyse the committed step in prostaglandin (PG) biosynthesis, prostaglandin endoperoxide H synthase (PGHS)-1 and PGHS-2 (Funk et al. 1991, Kujubu et al. 1991, O’Banion et al. 1991, Xie et al. 1991). The structure of each gene has been characterised and a 60% identity exists between the deduced amino acid sequences of the two human PGHS genes. PGHS-1 has been considered the constitutively expressed form of the enzyme in most tissues whereas PGHS-2 expression is induced by inflammatory cytokines, hormones and growth factors (reviewed in Smith & Dewitt 1995). PGHS-2 is considered, by virtue of its rapid synthesis and induction, an immediate early gene


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Cell culture and treatments

Amnion-derived WISH cells (Hayflick 1961) were maintained in a 50:50 mixture of DMEM and Ham’s-F12 medium plus 10% heat-inactivated FCS at 37 °C in a 95% air/5% CO2 atmosphere. Medium was supplemented with 60 µg/ml penicillin and 100 µg/ml streptomycin. WISH cells were grown to confluence in 6 cm tissue culture dishes for RNA and protein studies, 24-well plates for RIA and T75 Nunc flasks for the nuclear run-on transcription studies. At confluence, WISH cells were treated with 50 ng/ml TNF-α or vehicle for the indicated times.

Prostaglandin (PG)E2 radioimmunoassay (RIA)

WISH cells were treated with 50 ng/ml TNF-α for the times indicated and the medium was removed from cells and stored at −20 °C until use. PGE2 production was measured using a sensitive and specific RIA on media samples as described previously (Lundin-Schiller & Mitchell 1991). Cellular protein concentration was determined by the bicinchoninic acid (BCA) method (Redinbaugh et al 1986) calibrated against BSA. These experiments were repeated three times (n=3 wells per treatment) and are expressed as pg/µg cellular protein ± s.e.m.

Isolation of total RNA

Total cellular RNA was isolated according to the method of Chomczynski & Sacchi (1987). A total of 0.8 ml GTC (4 M guanidine thiocyanate containing 25 mM sodium citrate, pH 7.0, 0.5% Na–N-lauryl sarcosine and 0.1 M β-mercaptoethanol) was added to the WISH cells and the lysate was collected into 2.0 ml Eppendorf tubes. Forty microlitres 2.0 M sodium acetate, pH 4.0 were added. After mixing, 0.8 ml water-saturated phenol and 160 µl sodium citrate, pH 7.0, 0.5% Na–N-lauryl sarcosine and 0.1 M β-mercaptoethanol was added and vortexed until homogenous. Samples were placed on ice until phase separation was apparent. Samples were centrifuged at 10 000 g and the aqueous layer was removed into an equal volume of isopropanol. RNA was maintained at −20 °C for 1 h and centrifuged for 10 min at 10 000 g. Pellets were washed in ice-cold 75% ethanol and resuspended in 100 µl de-ionised formamide (DIF). RNA concentration was determined by measuring the absorbance at 260 nm.

mRNA half-life analysis

WISH cells were grown to confluence and TNF-α was added to the cells at a final concentration
of 50 ng/ml. After 1 h of TNF-α treatment, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) was added to the cells at a final concentration of 65 µM. At 0, 0.5, 1, 2, 4 and 8 h post-DRB addition, medium was removed and total RNA was isolated. Control cells were treated with ethanol carrier at a final concentration of 0.5%.

**Northern blot analysis**

Samples containing 15 µg total RNA were combined with an equal volume of RNA loading solution (40 mM MOPS, 2 mM sodium acetate, 2 mM EDTA, 12% w/v formaldehyde, 10% v/v glycerol, 0.1% bromophenol blue and 0.1% xylene cyanol), heated for 10 min at 65 °C, placed on ice, and loaded onto a 1% agarose gel containing 20 mM sodium phosphate, pH 7.2, 1 mM EDTA. The gel was removed and washed three times in distilled water. Staining with ethidium bromide and UV visualisation were used to assess RNA integrity and equivalence of loading. The gel was then incubated with shaking in 50 mM NaOH for 15 min and subsequently neutralised in 250 mM sodium phosphate pH 6.5 for 15 min. RNA was transferred to GeneScreen Plus by standard capillary transfer protocols using 25 mM sodium phosphate, pH 6.5. RNA was transferred to nitrocellulose and the Amersham Enhanced Chemiluminescence (ECL) system. Visualisation of the bands was accomplished by utilising a streptavidin–HRP conjugate (1:3000) and rabbit IgG (1:2000) was used as a secondary antibody. Completeness of transfer was determined by UV illumination. The positions of the 28S and 18S rRNA bands were marked and the blot was UV-irradiated with 1.2 × 10⁵ µJ in a Stratagene Stratalinker.

The membrane was prehybridised for between 1 and 2 h at +3 °C in a solution containing 50% formamide, 250 mM NaCl, 25 mM sodium phosphate, pH 7.2, 1 mM EDTA, 100 µg/ml salmon testis DNA and 7% SDS (Amasino 1986). Random-prime labelled 2.0 kb EcoRI/XbaI fragment of PGHS-2 (Funk et al. 1991) or the 1.8 kb EcoRI/ApaI fragment of the PGHS-2 isoform (Hla & Neilson 1992) cDNA (>10⁹ c.p.m./µg) was added to a final concentration of 10 ng/ml. Hybridisation was carried out under identical conditions for 16 h in a Techne hybridisation oven. Blots were washed two times in each of the following Northern wash solutions at +3 °C for 20 min. Wash 1: 2 × SSC, 0.5% SDS, Wash 2: 50 mM sodium phosphate pH 7.2, 0.5% SDS and 1 mM EDTA, Wash 3: 50 mM sodium phosphate pH 7.2, 5% SDS and 1 mM EDTA. The relative radioactivity in the resulting bands was quantitated on a Packard InstantImager. For half-life analysis, the results were plotted on a log relative level vs time scale. Blots were stripped in a 2% glycerol solution for 10 min at 80 °C and then re-probed for PGHS-1 utilising the above procedures.

**Immediate early gene analysis**

Confluent WISH cells were pre-treated with 10 µg/ml cycloheximide (CHX) or vehicle for 30 min. At t=0 (30 min after CHX addition), RNA was isolated from untreated cells or cells which had been treated with CHX for 30 min. TNF-α was then added to the remaining CHX- and vehicle-treated cells. At t=60 min, total RNA was isolated from cells treated with CHX only, TNF-α only, and CHX+TNF-α. PGHS-1 and -2 expression was assessed by Northern blotting as described above.

**Immunoblot analysis**

Confluent WISH cells were treated with either 50 ng/ml TNF-α or vehicle for the indicated times. Cells were washed twice with ice-cold PBS and lysed under hypotonic conditions (14 mM Na₂HPO₄, 5 mM NaH₂PO₄, 3 mM NaCl, 4 mM EDTA, 0.8 mg/ml benzamidine and 10 µg/ml PMSF, pH 7.4) and then centrifuged at 10,000 g to remove nuclei and mitochondrial fractions. The resulting supernatant was centrifuged at 100,000 g to isolate the microsomal fraction. Forty micrograms microsomal protein were separated on a 10% SDS–PAGE gels, transferred to nitrocellulose and probed with: (1) 1:1000 dilution of rabbit anti-human-TNF-α antisera (#410, University of Utah) in BLOTTO (PBS, 0.2% thimerasol and 1% non-fat dry milk powder). This antisera has been shown previously to be specific for the PGHS-2 isoform (Trautman et al. 1996). Biotinylated goat anti-rabbit IgG (1:2000) was used as a secondary antibody. Visualisation of the bands was accomplished by utilising a streptavidin–HRP conjugate (1:3000) and the Amersham Enhanced Chemiluminescence (ECL) system.

**Isolation of WISH cell nuclei**

WISH cells were grown to confluence in Nunc T75 flasks, medium was replaced and cells were allowed to grow for 4 h. TNF-α (50 ng/ml) or vehicle was added to the cells. At 40 min after TNF-α addition, cells were washed two times with ice-cold PBS, collected by scraping, and pelleted at 500 g. The cellular pellet was resuspended in 4 ml hypotonic N–P40 lysis buffer (10 mM Tris pH 7.5, 10 mM NaCl, 3 mM MgCl₂ and 0.5% N–P40) with vortexing. Nuclei were collected at 500 g and the supernatant was replaced with 4 ml hypotonic N–P40 lysis buffer. Nuclei were pelleted and an
aliquot was analysed microscopically to determine the extent of whole cell contamination of the nuclear preparation. Nuclei were counted and then resuspended in 200 µl glycerol storage buffer (50 mM Tris pH 8.0, 40% glycerol and 5 mM MgCl₂), frozen in liquid nitrogen and stored at −70 °C until use.

**Nuclear run-on transcription**

To the nuclei were added an equal volume (200 µl) of reaction buffer (10 mM Tris pH 8.0, 5 mM MgCl₂ and 300 mM KCl) containing 400 units RNasin/ml and 100 mM rCTP, rGTP and rATP. Twenty microlitres [α-32P]UTP (200 µCi) were added and the nuclei were incubated at 30 °C with shaking for 30 min. One hundred units DNase I were added and incubated at 30 °C with shaking for 10 min. One volume digestion buffer (100 mM Tris pH 8.0, 50 mM EDTA, 2% SDS, 100 µg/ml yeast tRNA and 400 µg/ml proteinase K) was then added and incubated at 30 °C for an additional 45 min. RNA was extracted with one volume of water-saturated phenol and the supernatant was combined with an equal volume of isopropanol and 100 µl 10 M ammonium acetate and then stored at −20 °C for at least 1 h. RNA was pelleted and resuspended in 400 µl GTC. An equal volume of isopropanol was added and kept at −20 °C for at least 1 h. RNA was pelleted and resuspended in 100 µl DIF. A 2 µl aliquot of the labelled RNA was counted in a Packard Scintillation counter. Hybridisation was performed with 5 × 10⁶ c.p.m./ml labelled RNA.

Membranes for hybridisation were prepared by slot-blotting 10 µg cDNA for PGHS-2, PGHS-1, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Tso et al. 1985) and pBS(SK-) onto a nitrocellulose membrane. The plasmids were linearised with the appropriate restriction enzyme(s). A 1/10 volume of 3 M NaOH was added to the restriction digest and incubated at 37 °C for 30 min. The reaction was then neutralised by the addition of one volume 2 M ammonium acetate. Once blotted, the membrane was UV-crosslinked as described previously. Membranes were prehybridised and subsequently hybridised for 2 days at 43 °C. Membranes were washed as described earlier and quantitated on a Packard InstantImager.

**Statistical analysis and data expression**

Measurements of PGE₂ production (pg/µg cellular protein) are shown as means ± standard error. Statistical significance was determined by ANOVA followed by a post-hoc Dunnett’s test. Significance was set at a P value of 0.05.

**RESULTS**

**Stimulation of PGE₂ biosynthesis in WISH cells by TNF-α**

Concentration–response experiments indicated that the effects of TNF-α on WISH cell PGE₂ production are near maximal at 50 ng/ml (data not shown). Figure 1 shows the temporal induction of
WISH cell PGE$_2$ production in response to the addition of 50 ng/ml TNF-$
$-$\alpha$. No significant increases in PGE$_2$ production were detected in the first hour after treatment with TNF-$\alpha$. However, 2 h after TNF-$\alpha$ addition, PGE$_2$ biosynthesis increased approximately 3-fold when compared with control cells (2·92 ± 0·19 vs 0·86 ± 0·11) and continued to increase approximately 180-fold by 24 h (60·90 ± 9·28 vs 0·33 ± 0·028).

**TNF-$\alpha$-induced rapid and bi-phasic induction of PGHS-2 mRNA expression**

We utilised the same time-course conditions to determine if the increase in PGE$_2$ production was associated with an increased expression of the PGHS-2 gene. Northern blot analysis of total RNA isolated from WISH cells stimulated by TNF-$\alpha$ showed that the PGHS-2 mRNA was rapidly synthesised and increased 6-fold within 30 min (Fig. 2A; lane 2) whereas in control cells the PGHS-2 mRNA was nearly undetectable (Fig. 2A; lanes 8–14) at all time points studied. The expression of the PGHS-2 mRNA was greatest at 1 h, demonstrating a 12-fold increase over control (lane 3). By 2 h, the PGHS-2 mRNA had returned to its low basal expression level (lane 4). At 8 h after TNF-$\alpha$ addition, the PGHS-2 mRNA was re-induced and remained elevated until at least 16 h (lanes 6 and 7). The blot was stripped and re-probed for the PGHS-1 mRNA. No change in the levels of the PGHS-1 mRNA was observed.

**TNF-$\alpha$-stimulated PGHS-2 transcription in WISH cells**

We determined the mechanism by which TNF-$\alpha$ induced rapid production of the PGHS-2 mRNA. WISH cell nuclei were isolated from cells that had been treated with TNF-$\alpha$ or vehicle for 40 min. Results of the nuclear run-on assay (Fig. 3) indicated a 20-fold increase in PGHS-2 transcription when compared with unstimulated cells. These results also show that the transcription rate of the PGHS-2 gene is very low in unstimulated cells which agrees with the low levels of the PGHS-2 mRNA detected in control cells (Fig. 2; lane 1). PGHS-1 transcription was only negligibly increased. GAPDH transcription was unchanged.

**Rapid decay of PGHS-2 mRNA in WISH cells**

TNF-$\alpha$ stimulated a rapid increase in PGHS-2 expression, however, the mRNA returned to unstimulated levels within 2 h of TNF-$\alpha$ addition. We wanted to determine if the rapid disappearance of the PGHS-2 message was due to rapid
cytoplasmic decay of the mRNA. Figure 4 shows the decay profile of the PGHS-2 mRNA after stimulation of WISH cells with TNF-α. One hour after TNF-α addition, the RNA polymerase II-specific transcriptional inhibitor DRB was added to the plates at a final concentration of 65 µM. Total RNA was then isolated and probed for PGHS-2 or -1 (Fig. 4A). The PGHS-2 mRNA decayed very rapidly within the initial 2 h after DRB addition. The apparent half-life of the PGHS-2 mRNA under these conditions was estimated to be 1 h (Fig. 4C). The decay profile showed that the PGHS-2 mRNA exhibited a bi-phasic pattern of degradation as the mRNA decayed at a slower rate after 2 h. The addition of ethanol carrier to the control cells had no effect on the expression of the PGHS-2 mRNA which exhibited a similar profile to that seen in Fig. 2 (Fig. 4A; lanes 7–11). In both instances, the PGHS-2 mRNA returned to unstimulated levels within 2 h of TNF-α addition. The PGHS-1 mRNA was very stable and did not decay during the time studied (Fig. 4A).

In WISH cells, TNF-α stimulated PGHS-2 mRNA expression within 30 min. As this up-regulation did not appear to occur in a time frame which required the synthesis of nuclear factors, we studied the effect of the protein synthesis inhibitor, CHX on PGHS-2 mRNA expression. Figure 5 shows that pre-treatment of cells for 30 min with 10 µg/ml CHX did not result in elevated PGHS-2 expression. Cells maintained on CHX for 90 min demonstrated a 3-fold increase in PGHS-2 mRNA. However, when cells were treated with CHX for 30 min and then stimulated with TNF-α, PGHS-2 expression was increased greater than 2-fold over that of TNF-α alone (25-fold vs 12-fold). Therefore, in the presence of a protein synthesis inhibitor, PGHS-2 mRNA production was still induced by TNF-α. This property has been previously demonstrated for immediate early genes (Muller et al. 1984).

**DISCUSSION**

This study examined the mechanism by which TNF-α stimulates the production of PGs in amnion-derived WISH cells. Using TNF-α at a final concentration of 50 ng/ml we determined that PG biosynthesis is stimulated within 2 h of TNF-α administration and increased in a time-dependent manner through a 24-h time period. In WISH cells, TNF-α has been previously shown to induce PGHS-2 expression while PGHS-1 expression remains unchanged (Perkins et al. 1996). In these studies, however, a direct transcriptional role of TNF-α was not established. Also, the dynamics of PGHS-2 mRNA expression differed from that presented herein.
In the present study, the expression of both the inducible and constitutively expressed forms of PGHS demonstrated very different profiles. The expression of the constitutive isoform, PGHS-1, was unaffected by the addition of TNF-α. However, PGHS-2 mRNA expression was induced within 30 min and increased rapidly to levels 12-fold above the control within 1 h which suggested a transcriptional induction of PGHS-2 gene by TNF-α. Of particular interest was a second up-regulation of PGHS-2 expression which occurred at 8 h after TNF-α addition and which continued for up to 16 h. This bi-phasic expression of PGHS-2 mRNA in response to TNF-α has not been previously reported and may be a unique characteristic of WISH cells which could possibly represent an

**FIGURE 4.** PGHS-2 mRNA decays rapidly in WISH cells treated with TNF-α. Confluent plates of WISH cells were treated with 50 ng/ml TNF-α for 1 h. The RNA polymerase II transcriptional inhibitor, DRB, was then added at a final concentration of 65 μM. Control cells were treated with ethanol at a final concentration of 0.5%. Total RNA was isolated at the indicated times post-DRB addition. (A) Northern blots were probed for PGHS-2 and then stripped and re-probed for PGHS-1. (B) Equivalence of loading was assessed by ethidium stain. (C) Decay profile of the PGHS-2 mRNA in TNF-α-treated WISH cells. mRNA half-life was determined by plotting imager units (c.p.m.) on a log relative mRNA level vs time scale and is representative of two separate experiments.
autocrine induction of PGHS-2 expression. However, this second induction of PGHS-2 expression does not seem to be due to endogenously produced PGs (Pilbeam et al. 1995) as inclusion of PGHS inhibitors (ibuprofen or NS–398) at concentrations that effectively inhibit PGHS activity and subsequent PG production do not affect the secondary response of the PGHS-2 gene to TNF-α (W R Hansen & M D Mitchell, unpublished observations). Therefore, auto-induction by PGs produced by the WISH cells seems to be an unlikely explanation for this phenomenon. It is possible that the dynamics of TNF-α receptor expression (Stewart & Marsden 1995) and the presence of residual bioactive TNF-α may explain the second induction of PGHS-2 expression at the 8- and 16-h time point.

The PGHS-2 gene is transcribed at a very low level in control cells. However, TNF-α induces a 20-fold increase in PGHS-2 transcription by 40 min which is most likely responsible for the large accumulation of the mRNA in Northern blots by 1 h. PGHS-1 transcription was affected only slightly by the addition of TNF-α. Of particular interest, however, is the difference in basal transcription rate of the PGHS-1 and -2 genes. The difference in basal transcription rates could be partially responsible for the differences in the respective PGHS mRNAs present in WISH cells in the unstimulated state.

There is extensive evidence that PGHS-2 is transcriptionally regulated by various stimuli (reviewed in Smith & Dewitt 1995). In the rat preovulatory follicle, a C/EBPβ element in the PGHS-2 promoter is required for the transcriptional up-regulation of PGHS-2 in response to gonadotrophic hormone (Sirois et al. 1993). In vascular endothelial cells, lipopolysaccharide (LPS) and 12-O-tetradecanoyl-13-acetate required a C/EBPβ response element within the PGHS-2 promoter (Inoue et al. 1995). Basic fibroblast growth factor stimulated PGHS-2 transcription in MC-3T3E1 cells and exerted its effects through the first 371 nucleotides of the mouse PGHS-2 promoter (Kawaguchi et al. 1995).

The transcriptional effects of TNF-α have been characterised in many cell types and there are several mechanisms by which it activates transcription. The stimulation of E-selectin expression by TNF-α requires a CRE/ATF element (De Luca et al. 1994). The NF–IL6 (C/EBP–β) and AP-1 sites in the TSG-6 promoter are both required for TNF-α activation (Klampfer et al. 1994). Induction of urokinase expression in a squamous cell carcinoma cell line required both AP-1 and PEA3 transcription factor binding sites (Lengyel et al. 1995). Of particular interest, the need for NF–κB consensus domains in many promoters for the activity of TNF-α (Grilli et al. 1993). However, the activation of PGHS-2 transcription in WISH cells by TNF-α does not seem to be dependent on the presence of NF–κB sites in the PGHS-2 promoter (W R Hansen & M D Mitchell, unpublished observations) The human PGHS-2 promoter contains many of the consensus sequences that could be utilised to convey the transcriptional effects of TNF-α (Tazawa et al. 1994).

**Figure 5.** PGHS-2 is an immediate early gene in WISH cells. Confluent WISH cells were either pre-treated with 10 µg/ml CHX or vehicle for 30 min. At t=0 (30 min after CHX addition), RNA was isolated from untreated plates (C) or CHX plates for 30 min (CHX30). TNF-α was then added to CHX- and vehicle-treated plates. At t=60 min, total RNA was isolated from CHX only (CHX90), TNF-α only (TNF), and CHX+TNF-α (THF+CHX) plates. Blots were probed for PGHS-2 and then stripped and re-probed for PGHS-1. Equivalence of loading was assessed by ethidium stain. The results are representative of two individual experiments.

**Figure 6.** Immunoblot analysis of PGHS-2 protein in TNF-α-stimulated WISH cells. Confluent WISH cells were treated with either 50 ng/ml TNF-α or vehicle for the indicated times. Forty micrograms microsomal protein were separated on 10% SDS–PAGE gels and probed with a 1:1000 dilution of rabbit anti-human PGHS-2 antisera. Biotinylated goat anti-rabbit IgG (1:2000) was used as a secondary antibody. Visualisation of the bands was accomplished by utilising a streptavidin–HRP conjugate (1:3000) and the Amersham ECL system. The figure is representative of two separate experiments.
The PGHS-2 mRNA decayed with an apparent half-life of 1 h in TNF-α-stimulated WISH cells in the initial 2 h of the experiment. The PGHS-2 3′-nontranslated region contains several copies of the canonical AUUUA sequence motifs which could explain the rapid disappearance of the PGHS-2 mRNA after TNF-α addition. However, from the time course studies, one would expect a more rapid estimate of the PGHS-2 mRNA half-life as the mRNA has returned to basal levels within 2 h after the addition of TNF-α. The use of transcriptional inhibitors often has aberrant effects on the stability of immediate early gene mRNAs and in some cases stabilise the mRNA against decay by inhibiting either deadenylation or actual decay of the mRNA. The RNA polymerase II-specific transcriptional inhibitor, DRB, which inhibits transcription by inhibiting phosphorylation of the C-terminal domain (CTD) of RNA polymerase II (Dubois et al. 1994), has been shown to impede mRNA degradation slightly but not to the same extent as actinomycin D (Chen et al. 1995). DRB, however, more closely approximates results from pulse–chase mRNA decay analysis (Harrold et al. 1991, Ross 1995) which does not rely on the use of transcriptional inhibitors. These aberrant effects on mRNA decay by transcriptional inhibitors could partially explain the bi-phasic pattern of PGHS-2 mRNA decay. Furthermore, the lack of PGHS-1 decay in these experiments suggests that the stability of the PGHS-1 mRNA and its relatively high transcription rate (when compared with PGHS-2) is responsible for the high constitutive expression of the PGHS-1 mRNA. This could explain the differences in PGHS-1 and -2 expression in WISH cells under normal growth conditions. Also, it is unlikely that a negligible increase in PGHS-1 transcription would be detectable given the constitutively high level of this mRNA as it is unknown if this event is of a temporally transient nature. The transcriptional activity of the PGHS genes has not been determined at later time points.

PGHS-2 displays the intrinsic characteristics of an immediate early gene in other cell types (Herschman 1991). Our data indicates that PGHS-2 exhibits these same properties in WISH cells. Given the proposed function of PGHS-2 in most cell types, rapid transcriptional activation without the requirement for protein synthesis would be integral for rapid cellular responses to mitogenic stimulation.

We found that TNF-α induced the production of PGHS-2 protein within 30 min although this did not appear to allow enough time for a significant increase in PGE2 production to occur. The levels of protein remained elevated until at least 16 h. This is an interesting finding as the levels of the protein remained high whilst the PGHS-2 mRNA had a discernible lag in synthesis for up to 7 h after the initial transcriptional increase. Furthermore, the rate of PGE2 secretion increased throughout the time studied suggesting that PGHS-2 was responsible for the levels of PGE2 in the culture medium. Our findings differ from reports which found that PGHS-2 protein in WISH cells was not detectable until 1 h after the addition of TNF-α (Perkins et al. 1996). The reason for this discrepancy is unknown, however, it is possible that the utilisation of different PGHS-2 antibodies could yield slightly differing results.

We have presented evidence that TNF-α stimulates bi-phasic synthesis of PGHS-2 mRNA in WISH cells and characterised the initial response. The actions of TNF-α are of great concern as the administration of TNF-α in utero causes foetal death in a murine model. Also, pretreatment with antibodies specific for TNF-α significantly reduced LPS-mediated foetal death (Silver et al. 1991). It is likely that intrauterine (in particular, amnion) PGHS-2 expression is stimulated by TNF-α by a mechanism similar to the findings we have presented. Therefore, it is possible that TNF-α produced during infection-associated preterm labour may promote a cascade of events in the amniotic layer of the placental membranes which includes induction of PGHS-2 expression and subsequent propagation of PGE2 production. These events may also involve the vigorous production of other cytokines and PGs which have been the proposed initiators of preterm labour (Karim & Devlin 1967, Dudley & Trautman 1994).

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