Heat Shock of Rabbit Synovial Fibroblasts Increases Expression of mRNAs for Two Metalloproteinases, Collagenase and Stromelysin

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Abstract. Two metalloproteinases, collagenase and stromelysin, are produced in large quantities by synovial fibroblasts in individuals with rheumatoid arthritis. These enzymes play a major role in the extensive destruction of connective tissue seen in this disease. In this study, we show that heat shock of monolayer cultures of rabbit synovial fibroblasts increases expression of mRNA for heat shock protein 70 (HSP-70), and for collagenase and stromelysin. We found that after heat shock for 1 h at 45°C, the mRNA expression for HSP-70 peaks at 1 h and returns to control levels by 3 h. Collagenase and stromelysin mRNA expression is coordinate, reaching peak levels at 3 h and returning to control levels by 10 h. The increase in mRNA is paralleled by an increase in the corresponding protein in the culture medium. 3 h of heat shock at a lower temperature (42°C) is also effective in inducing collagenase and stromelysin mRNAs. Concomitant treatment with phorbol myristate acetate (PMA; 10^{-8} or 10^{-9} M) and heat shock is not additive or synergistic. In addition, all-*trans*-retinoic acid, added just before heat shock, prevents the increase in mRNAs for collagenase and stromelysin. Our data suggest that heat shock may be an additional mechanism whereby collagenase and stromelysin are increased during rheumatoid arthritis and perhaps in other chronic inflammatory stress conditions.

CONNECTIVE tissue metalloproteinases are enzymes that are active at neutral pH, contain Zn^{++}, and have the ability to degrade the extracellular matrix (31, 39, 46). Recent work by our laboratory (21, 22) and others (23, 31) indicates that metalloproteinases comprise a multigene family whose members share considerable structural similarities (reviewed in reference 31). Two members of this gene family are collagenase and stromelysin. Collagenase (57,000 mol wt) is an enzyme that has the singular ability to initiate the breakdown of the interstitial collagens, types I, II, and III (31, 46), while stromelysin (55,000 mol wt) is an enzyme that degrades noncollagen matrix; e.g., proteoglycans, laminin, and fibronectin. It can also activate latent collagenase (31).

Modeling and remodeling of connective tissue by metalloproteinases occurs in a number of normal and disease states: wound healing, uterine resorption, and tumor invasion (reviewed in references 31, 46). Nowhere, however, is the impact of excess production of metalloproteinases more apparent than in rheumatoid arthritis, a chronic inflammatory autoimmune disease in which the fibroblasts (synovial cells) that line the joints secrete large quantities of collagenase and stromelysin. The result is rampant destruction of articular cartilage and bone (6, 27, 28).

Using a model system of rabbit synovial fibroblasts cultured in vitro, we and others have shown that cell stress induced by fusion of fibroblasts with polyethylene glycol (7), phagocytosis of crystals of monosodium urate monohydrate (26), or treatment with PMA (8, 13, 21) increases the synthesis of mRNAs for collagenase and stromelysin. Heat shock, another form of cellular stress, has been associated with an increase in synthesis of a family of peptides known as heat shock proteins (HSPs)[1] (5, 16, 41). Although the function of HSPs has not been determined, investigators have hypothesized that these proteins may be involved in embryological development, cell growth and proliferation, and in a survival mechanism referred to as the SOS (emergency repair) response (reviewed in references 5, 16, 41). In addition, several of the heat shock proteins 70 (HSP-70s) may participate in the translocation of proteins through stages of the protein maturation process inside the cell (33). An HSP-90 is part of the glucocorticoid receptor and plays a role in the transduction process to produce glucocorticoid effects (40).

As part of the inflammation associated with rheumatoid arthritis, local increases in temperatures may occur within joints (27, 28). We proposed that the stress of increased temperature might increase expression of collagenase and stromelysin, and therefore heat shock may play a role in perpetuating the joint destruction seen in rheumatoid arthritis.

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1. *Abbreviations used in this paper*: HSE, heat shock element; HSP, heat shock protein; HSP-70, heat shock protein 70.

Materials and Methods

Cell Culture

New Zealand White rabbits (4–6 wk old; Snelling Rabbitry, Claremont, NH) were killed and synovial tissue was removed and dissociated into a single cell suspension with bacterial collagenase and trypsin (10, 15, 19). Primary cultures of rabbit synovial fibroblasts were plated in DME (Gibco Laboratories, Grand Island, NY), supplemented with 20% FCS (Gibco Laboratories), and grown to confluency at 37°C in a humidified atmosphere containing 5% CO_2. Cultures were passed three times in DME containing 10% FCS, antibiotics, and glutamine to establish sufficient numbers of cells. For experiments, the fibroblasts were used between passages three and six. These cells secrete minimal amounts of collagenase and stromelysin in the unstimulated state.

Heat Shock Protocol

Rabbit synovial fibroblasts were grown to 80% confluency in 150-mm-diam culture dishes as described above. Just before heat shock, culture dishes were tightly wrapped in parafilm to minimize loss of CO_2 and then the cultures were placed in a New Brunswick Scientific Co. (Edison, NJ) incubator for heat shock: 1 h at 45°C, or 3 h at 42°C. After heat shock, the dishes were unwrapped and returned to 37°C, 5% CO_2, and RNA was harvested at various time points (see below). As controls, some cultures remained at 37°C in 5% CO_2 during the time of heat shock, while other control cultures were wrapped in parafilm and placed at 25°C in the tissue culture hood to control for possible effects of decreased CO_2. We found that three culture dishes (150 mm diam) for each experimental point provided sufficient quantities of RNA for analysis: control plates yielded an average of 220 µg of whole cell RNA/plate, while the experimental plates yielded an average of 190 µg of whole cell RNA/plate. Except where noted otherwise, all experiments were performed two or three times.

Northern Blot Analysis

Whole cell RNA was isolated, as described previously (15, 17), and 25 µg/lane was electrophoresed on a 1% agarose/6% formaldehyde gel for Northern blot analysis (21, 25, 26, 30). After electrophoresis, the RNA was transferred to Gene Screen Plus (New England Nuclear, Boston, MA) and hybridized with ^{32}P-labeled cDNA probes for either Drosophila HSP-70 (kindly provided by Dr. J. Zurlo, Dartmouth Medical School, Hanover, NH), or rabbit collagenase or stromelysin. Both the rabbit collagenase (26) and stromelysin (21) cDNAs were isolated and characterized in this laboratory. Stringent wash conditions were used: two washes in 2× SSC + 0.5% SDS at 65°C for 45 min followed by one wash in 0.5× SSC + 0.5% SDS for 45 min at 65°C. The HSP-70 cDNA hybridizes with a 2.4 kb mRNA, collagenase cDNA hybridizes with a 2.0 kb mRNA, and stromelysin cDNA hybridizes with a 1.9 kb mRNA. A cDNA clone for bovine pyruvate kinase (a gift from Biogen Co., Cambridge, MA) which detects a mRNA of 2.4 kb was used as a "housekeeping gene" to control for variations in the amount of RNA loaded per lane. cDNA probes were radiolabeled with [^{32}P]dCTP by the oligolabeling technique of Feinburg and Vogelstein (20). The Northern blots were exposed to XAR-5 film (Eastman Kodak Co., Rochester, NY) and densitometry was performed on selected audioradiographs to compare levels of induction.

Immunoprecipitation

Cultures in 100-mm-diam culture dishes were heat shocked in DME + 10% FCS at 42°C for 3 h. Immediately after heat shock, the cells were transferred to 5 ml of methionine-free medium and incubated for 30 min at 37°C to deplete the intracellular pool of methionine (32). The medium was then replaced with fresh 5 ml of methionine-free medium and 300 µC [^{35}S]methionine was added. After 5 h, medium was harvested and [^{35}S]methionine-labeled collagenase was measured by immunoprecipitation of 4 ml of culture medium with monospecific antibodies to rabbit collagenase, SDS-PAGE, and autoradiography (10, 32).

Results

HSP-70 mRNA Expression

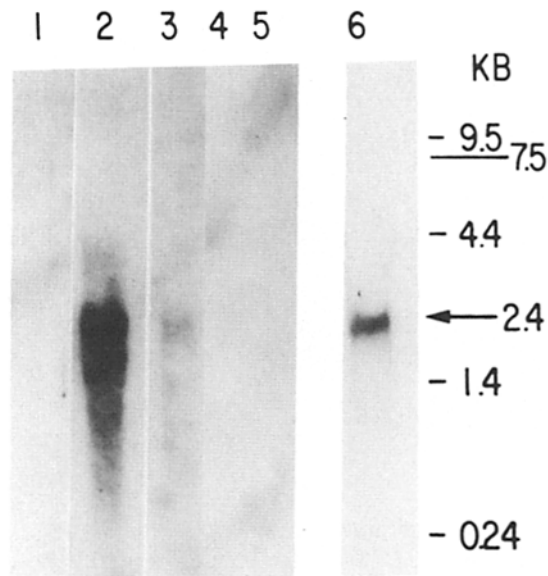Rabbit synovial fibroblasts were heat shocked at 45°C for

Figure 1. Time course of HSP-70 mRNA expression in rabbit synovial fibroblasts. Rabbit synovial fibroblasts at 80% confluency and cultured in DME + 10% FCS were heat shocked for 1 h at 45°C and harvested at the indicated time points (lanes *1–5*). Another group of rabbit synovial fibroblasts were treated for 48 h with PMA (lane *6*) at 37°C and RNA was then harvested. Northern blot analysis was performed with 25 µg/lane of whole cell RNA and probed with a [^{32}P]oligo-labeled cDNA probe for *Drosphila* HSP-70. Exposure time of blot to x-ray film was 24 h. Lane *1*, untreated control (37°C), harvest RNA at 3 h. Heat shock, harvest RNA at 1, 3, 10, and 24 h (lanes *2–5*, respectively); lane *6*, PMA for 48 hours (37°C) and harvest RNA.

1 h, returned to 37°C, and whole cell RNA was harvested at 1, 3, 10, and 24 h (Fig. 1). By Northern blot analysis and densitometry, we found that heat shock results in a 14-fold increase in mRNA that is detected by a cDNA probe for *Drosophila* HSP-70 (Fig. 1, lane 2). This increase peaks at 1 h and returns to control levels by 10 h (Fig. 1, lane 4). In addition, we found that treatment for 48 h with PMA (10^{-8} M) induces a fivefold increase in HSP-70 mRNA. These results are consistent with previous reports showing an increase in the activity of HSPs after treatment with PMA, UV light, or mitomycin C (4, 44). In addition, they suggest that our heat shock protocol and culture system of rabbit synovial fibroblasts are suitable for the study of the regulation of mRNA expression by heat shock.

Heat Shock Induces Metalloproteinase mRNA Expression

Levels of mRNA expression for two metalloproteinases, collagenase and stromelysin, were determined at intervals after heat shock in the same experiment as described above. The time course of induction of mRNA for these two metalloproteinases was determined by Northern blot analysis (Fig. 2). The figure shows coordinate induction of both mRNAs which peaked at 3 h (Fig 2, lanes *3*) and returned to control levels by 10 h (Fig. 2, lanes *5*).

In addition, we found that heat shock induces these metalloproteinases in a dose-dependent fashion: a 60-min exposure to 45°C induces twice as much metalloproteinase
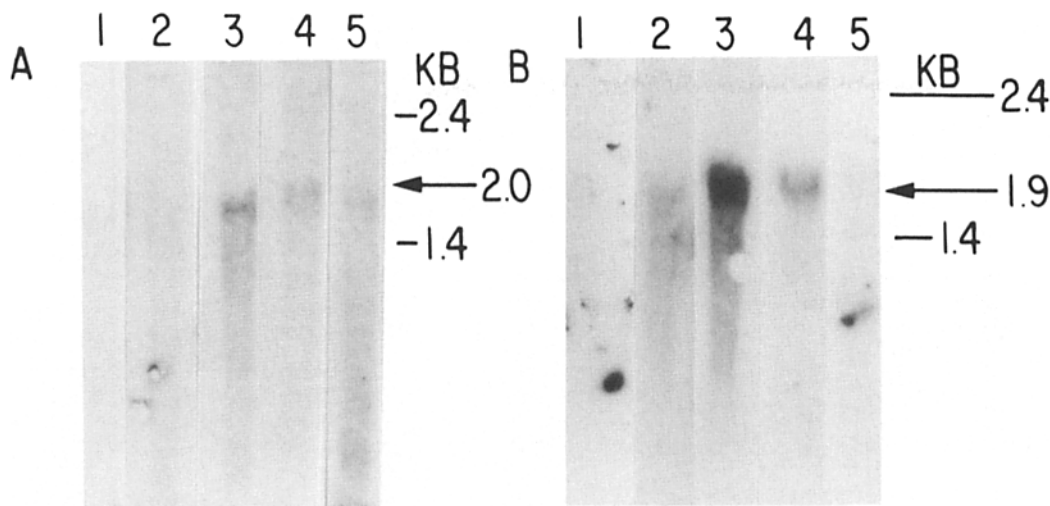
Figure 2. Time course of metalloproteinase mRNA expression. Rabbit synovial fibroblasts at 80% confluency and cultured in DME + 10% FCS were heat shocked for 1 h at 45°C, returned to 37°C, and harvested at indicated time points. Northern blot analysis was performed with 25 μg/lane of whole cell RNA and probed with [^{32}P]oligo-labeled cDNA probes for either collagenase (*A*) or stromelysin (*B*). Exposure time of blots to x-ray film was 24 h for collagenase and 16 h for stromelysin. Lanes *1*, untreated control (37°C), harvest RNA at 3 h; Heat shock, harvest RNA at 1, 3, 6, and 10 h (lanes *2–5*, respectively).

mRNA as a 30-min heat shock at this same temperature (data not shown). In other experiments, rabbit synovial fibroblasts were heat shocked at a somewhat lower temperature (42°C) for a longer period of time; i.e., 3 h (see Fig. 4, below). These conditions were also effective in increasing collagenase and stromelysin mRNA expression, whereas heat shock at 42°C for 1 h was not (data not shown).

To determine that an increase in mRNA was accompanied by an increase in protein in the culture medium, we used our monospecific antibody to collagenase (10, 32) to immuno-precipitate [^{35}S]methionine-collagenase from culture medium (Fig. 3). When the bands of immunoprecipitated [^{35}S]methionine-collagenase were quantified by scintillation counting (10, 31), we found that control cultures that were not heat shocked secreted 710 cpm, while cells subjected to heat shock produced 1,219 cpm of [^{35}S]methionine-collagenase. In several other experiments we consistently observed an increase in metalloproteinase, measured by immunoprecipitation, Western blotting, or visualization of total culture medium proteins by SDS-PAGE (10, 26) (data not shown). This qualitative correlation between mRNA and protein agrees with our previous studies on the appearance of collagenase mRNA and protein after stimulation of rabbit synovial fibroblasts with crystals of monosodium urate monohydrate (26).

Finally, heat shock, under the conditions we have described was not toxic to rabbit synovial fibroblasts, as revealed by the equivalent amounts of RNA recovered from treated and control plates (see Materials and Methods). This is in agreement with the nontoxic nature of heat shock on monocytes reported in other laboratories (35) as measured by trypan blue exclusion and [^{3}H]thymidine incorporation in cells.

Retinoic Acid Inhibits PMA and Heat Shock Induction of Metalloproteinase mRNA Expression

Treatment of fibroblasts with all-*trans*-retinoic acid (10^{-6}

M) antagonizes the induction by a number of agents of collagenase and stromelysin mRNAs and protein (8, 11, 12, 14, 18, 21). We determined whether retinoic acid could also antagonize the heat shock–induced increase in mRNAs for these metalloproteinases. Cells were heat shocked for 3 h at 42°C or treated with PMA (10^{-8} M) at 37°C in the presence
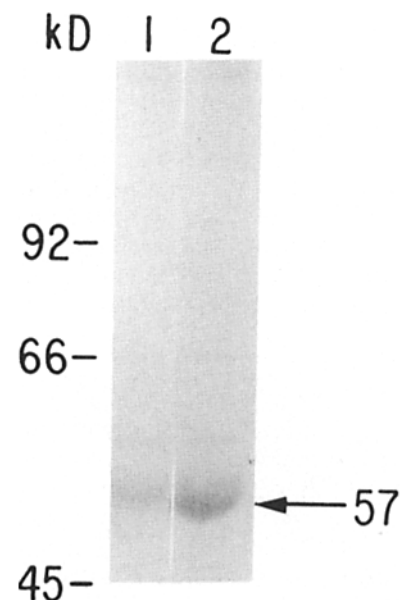

Figure 3. Immunoprecipitation of [^{35}S]methionine collagenase. Rabbit synovial fibroblasts, grown in DME + 10% FCS, were heat shocked for 3 h at 42°C. Both control cells and heat-shocked cells were then transferred to methionine-free medium with [^{35}S]methionine and incubated for 5 h at 37°C. Radiolabeled collagenase was immunoprecipitated with a monospecific antibody and visualized by SDS-PAGE and autoradiography. Lane *1*, Untreated control (no heat shock); lane *2*, heat shocked.
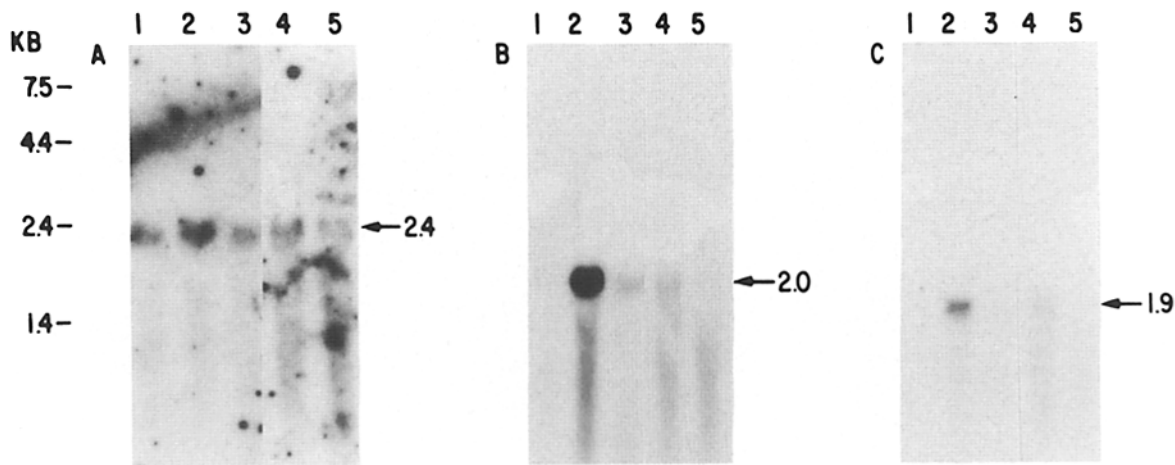
Figure 4. Effect of retinoic acid on phorbol ester and heat shock induction of metalloproteinase mRNA expression. Rabbit synovial fibroblasts at 80% confluency and cultured in DME + 10% FCS were heat shocked at 42°C or treated with PMA (10^{-8} M) for 3 h in the presence of all-*trans*-retinoic acid (10^{-6} M). The retinoic acid was added immediately before heat shock or treatment with PMA. After the heat shock or exposure to PMA, all cells were washed three times with HBSS, control medium without PMA or control medium containing retinoic acid was added to all cultures, and the cells were returned to 37°C. RNA was harvested 3 h later. Northern blot analysis was performed with 25 μg/lane of whole cell RNA and transferred to Gene Screen Plus, the RNAs were probed with [^{32}P]oligo-labeled cDNA probe for pyruvate kinase (*A*). The blot was stripped and reprobed with cDNA probe for collagenase (*B*) and the procedure was repeated and reprobed with cDNA for stromelysin (*C*). Exposure time of the blots to x-ray film was 16 h for pyruvate kinase, 16 h for collagenase, and 12 h for stromelysin. Lanes *1*, untreated control (37°C) for 3 h; lanes *2*, PMA (37°C) for 3 h; lanes *3*, PMA + all-*trans*-retinoic acid (37°C) for 3 h; lanes *4*, heat shock (42°C) for 3 h; lanes *5*, heat shock (42°C) + all-*trans*-retinoic acid for 3 h.

or absence of all *trans*-retinoic acid. Cells were then washed and medium was replaced with medium without PMA, but containing retinoic acid. Cells were returned to 37°C and whole cell RNA was harvested 3 h later. In this experiment, densitometric analysis revealed that heat shock increased collagenase mRNA approximately twofold over control, while stromelysin mRNA was increased fourfold (see Discussion). We found that all-*trans*-retinoic acid prevented the increase in collagenase and stromelysin mRNA expression (Fig. 4, *B* and *C*). It is important to point out that during the same experiment, pyruvate kinase remains relatively unchanged regardless of treatment (Fig. 4 *A*). A densitometric comparison of mRNA for this gene revealed a relative intensity of 0.88 ± 0.3 OD. This suggests that this gene is an appropriate "housekeeping gene" that does not change dramatically during heat shock in rabbit synovial fibroblasts.

Effect of Combined Heat Shock and PMA on Induction of Metalloproteinase mRNA Expression

PMA is a potent inducer of collagenase and stromelysin mRNAs (8, 11, 13, 15, 21, 24) and we wanted to determine whether combined treatment with PMA and heat shock was either additive or synergistic. For this experiment, cells were treated with PMA for 1 h at 37°C, were heat shocked for 1 h at 45°C, or were subjected to heat shock (1 h at 45°C) in the presence of PMA. Medium without PMA was then replaced on all cultures and the cells were returned to 37°C for 3 h and then harvested. The results (Fig. 5) show that heat shock of rabbit synovial fibroblasts in the presence of PMA (10^{-9} or 10^{-8} M) did not increase collagenase or stromelysin mRNA expression (Fig. 5, lanes *6* and *7*) above treatment with PMA alone or above heat shock alone (Fig. 5, lanes *2*, *3*, and *5*). We also found that the room temperature control

cells held at 25°C for 1 h showed virtually no difference in the levels of collagenase and stromelysin mRNAs, relative to the control cells held at 37°C (Fig. 5, lanes *1* and *4*).

Densitometric scanning of the autoradiographs in this experiment revealed that heat shock increased collagenase and stromelysin mRNAs to the same extent; i.e., approximately sixfold. In a total of 12 experiments, we have noted that collagenase and stromelysin mRNAs increase two- to tenfold. In addition, although the time course of increase for collagenase and stromelysin is always coordinate, the magnitude of the response for each mRNA may vary within an experiment. As we have noted previously with PMA treatment (8, 13, 15), the variation in the magnitude of the increase in collagenase and stromelysin is typical of the responsiveness of monolayers of rabbit synovial fibroblasts. Possible reasons for this variability are discussed below.

Localization of PMA Element and Putative Heat Shock Element (HSE)

PMA is a potent inducer of metalloproteinase gene expression (2–4, 8, 11, 13, 15, 21–24) and a PMA-responsive element has been localized in the 5' flanking DNA of the collagenase (2–4, 22) and stromelysin (23) genes (see Table I). An HSE has been identified in the HSP-70 gene (34), as well as in other genes (42). In *Drosophila*, the HSE is located in the 5' flanking region of the HSP-70 gene −45 to −66 bp from the start site of transcription. In addition, an HSE has been identified in the rat heme oxygenase gene −273 bp from the start site of transcription (42). We, therefore, examined the 5' flanking DNA of the rabbit collagenase and stromelysin genes for putative HSEs, as shown in Table I. The putative HSEs are compared with a "stringent" nucleotide sequence (defined by Pelham [34] and Schlesinger [41]) and the
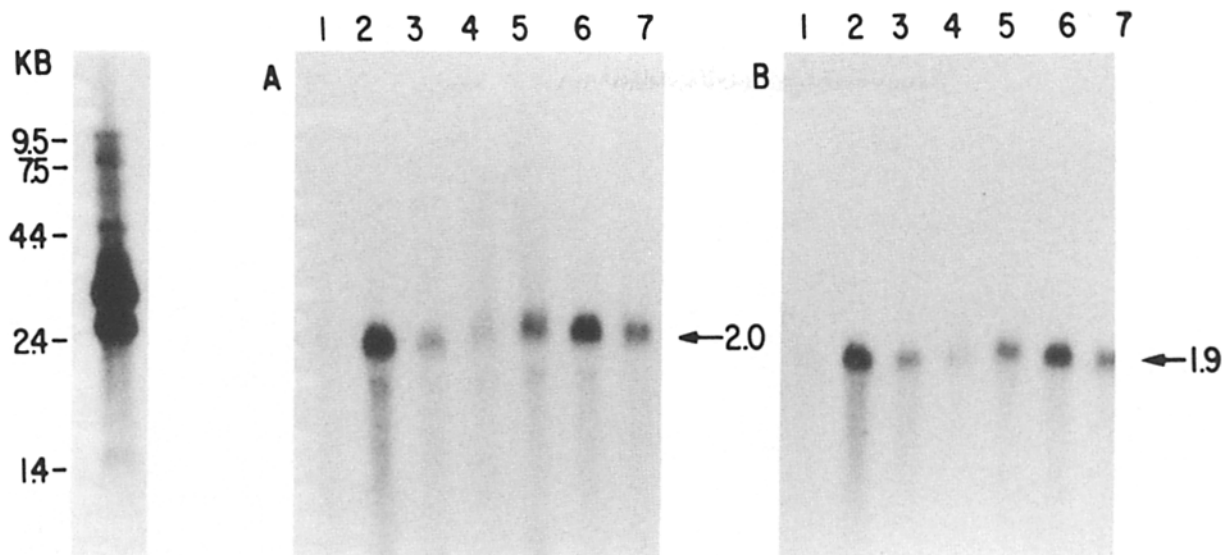
Figure 5. Effect of combined heat shock and phorbol ester on induction of metalloproteinase mRNA expression. Rabbit synovial fibroblasts at 80% confluency and cultured in DME + 10% FCS were treated with PMA (10^{-8} or 10^{-9} M) for 1 h at 37°C and/or heat shocked for 1 h at 45°C. Medium without PMA was replaced on all cultures, and the cells were returned to 37°C for 3 h when RNA was harvested. Northern blot analysis was performed with 25 μg/lane of whole cell RNA and after transfer to Gene Screen Plus, the RNAs were probed with [^{32}P]oligo-labeled cDNA probe for collagenase (*A*). The blot was stripped and reprobed with a cDNA probe for stromelysin (*B*). Exposure of blots to x-ray film was 18 h for collagenase and 15 h for stromelysin. Lanes *1*, untreated control (37°C), 1 h; lane *2*, PMA (10^{-8} M), 1 h (37°C); lane *3*, PMA (10^{-9} M), 1 h (37°C); lane *4*, untreated control (25°C), 1 h; lane *5*, heat shock (45°C), 1 h; lane 6, heat shock (45°C) + PMA (10^{-8} M), 1 h; lane *7*, heat shock (45°C) + PMA (10^{-9} M), 1 h.

mismatches are underlined. Note that one of the putative HSEs in the collagenase gene (−78) is similar to the location of the HSE for *Drosophila* HSP-70, while two putative HSEs in the stromelysin gene (−265 and −278) are similar to the location of the HSE in the rat heme oxygenase gene.

Discussion

In this study, we show that physical stress in the form of heat shock can increase the expression of collagenase and stromelysin mRNAs and protein. This expression was not enhanced by concomitant treatment with PMA, a potent inducer of metalloproteinases, but the heat shock induction could be antagonized by all-*trans*-retinoic acid, a compound known to inhibit metalloproteinase synthesis induced by a variety of agents (8, 11, 12, 14, 15, 18, 21).

Our data on the time course of induction of collagenase and stromelysin mRNAs by heat shock (45°C for 1 h or 42°C for 3 h) show a coordinate increase in both mRNAs. They peak by 3 h after heat shock, and return to control levels by 10 h. This increase in mRNA is accompanied by an increase in the secreted protein for these enzymes, measured by immunoprecipitation of [^{35}S]methionine-collagenase. In previous studies, we demonstrated that the time required for synthesis and secretion of collagenase is ∼45 min (32). Thus, although the increase in mRNA is closely linked to the increase in protein there is a slight lag (26). Furthermore, the increase in collagenase and stromelysin mRNAs and proteins in the culture medium is coordinate (11, 21, 24).

At this point, we do not completely understand the variation in the magnitude of induction of mRNA for collagenase and stromelysin seen with heat shock. We have long noted variation in the quantitative response of collagenase seen in

synovial fibroblasts. This variation is apparent whether polyethylene glycol (7, 8), urate crystals (26), or phorbol esters (8, 13) are used as the inducing agent. In the past we have attributed the variability to the fact that these cultures of rabbit synovial fibroblasts represent an outbred population with inherent genetic differences in their responsiveness. As we learn more about the cellular mechanisms involved in the induction of collagenase synthesis (9, 10), our understanding of the basis for these differences should increase.

As already mentioned, PMA is a potent inducer of collagenase, greatly increasing levels of collagenase mRNA and protein (8, 11, 13, 15, 21, 24). However, it is important to point out that when the rabbit fibroblasts were subjected to a 1-h treatment with PMA or to 1 h of heat shock (45°C), the magnitude of the metalloproteinase response was similar for both treatments (Fig. 4, *B* and *C*). Furthermore, combined treatment with PMA and heat shock was neither additive nor synergistic. These data suggest that induction of metalloproteinases by heat shock and by PMA may occur via similar mechanisms. It seems possible, for example, that PMA triggers an intracellular pathway that eventually results in the transcription factor, AP-1, binding to the PMA consensus sequence (5′-ATGAGTCAG-3′) and subsequently increasing the transcription of the collagenase gene (2–4, 22). It is also possible that another transcription factor (e.g., heat shock transcription factor), similar to those isolated from yeast and HeLa cells (43), may interact with the putative HSEs that we have identified in the DNA flanking the 5′ regions of the collagenase and stromelysin genes (Table I), thereby inducing these genes. The differential responsiveness sometimes seen in the magnitude of induction of mRNA for stromelysin and collagenase genes to heat shock may in part be due to the number and location of HSEs (29) in these

Table I.

Consensus sequence	Location	Mismatches
Collagenase		
Heat shock		
CTTGAAGAATGGAG	−1151	2
TTAGAACCTTTGAA	−955	3
CCAGGAAGTTCATG	−881	3
CATTCAAATTCTAG	−603	3
CCAGCAGAATCAAG	−358	3
CATGAAATTGCAAC	−78	3
PMA		
CATGAGTCAG	−77	
Stromelysin		
Heat Shock		
CCTGTATTTTAGAG	−473	3
CTTAAAAGTTCTGC	−356	3
CTAGTAAATTCTAG	−278	1
GTCAAATTTTCCAG	−265	2
PMA		
TGAGTCAAG	−65	

Localization of PMA and putative HSE consensus sequences in the 5′ flanking region of the collagenase and stromelysin genes for rabbit. Listed are the locations (5′ to start site of transcription), and nucleotide sequences for the PMA and putative HSE consensus sequences for rabbit collagenase and stromelysin genes. For the HSE consensus sequences, the number of mismatches is based on comparison with stringent, (CTNGAANNTTCNAG) HSE consensus sequences described by Pelham (34). Mismatched nucleotides are underlined.

genes (Table I). The number of PMA-responsive elements has been shown to be important for the PMA induction of metalloproteinases (2, 3).

Other investigators have shown that the heat shock response can induce heme oxygenase, an enzyme essential in heme catabolism, in rat glioma cells (42). These experiments are interesting for two reasons. First, in contrast to our studies with PMA, they found that heat shock was additive with hemin, another heme oxygenase inducer, implying two different mechanisms of induction for these enzymes. Second, their report documents that heat shock can induce proteins other than the "classical" HSPs, and thus they begin to suggest physiologic roles for the heat shock response. Further support for this concept is given by studies documenting that heat shock of HeLa cells increased the expression of the c-*fos* protooncogene (1). They postulate that the increase in c-*fos* mRNA may facilitate the reinitiation of the cell cycle during recovery from stress.

The concept that HSPs could be involved in the pathophysiology of inflammation has been proposed by Polla et al. (35–38). They investigated the possible effects that heat shock of monocytes may play in several inflammatory conditions. They found that temperatures of 41–45°C for 20 min resulted in an increase in a variety of HSPs, and that α-1,25-dihydroxyvitamin D_3 could protect the cells from thermal injury, perhaps by shielding the cells from oxidative damage by enzymes such as superoxide dismutase or catalase. As we have shown in this study, heat shock at a lower temperature for a longer period of time, 3 h at 42°C vs. 1 h at 45°C, is an effective inducer of metalloproteinases. This implies that a relatively low level of heat shock for a prolonged period (seen, for example, in chronic inflammation) may be a mech-

anism for increasing the expression of these enzymes in disease states.

The heat shock response in monocytes (35–38) and our work on the induction of metalloproteinases in fibroblasts by heat shock support the hypothesis that heat shock is one of several stress inducers in eukaryotic cells (5). Indeed, we have long suspected that induction of metalloproteinases was directly linked to stress, as evidenced by an increase in collagenase synthesis after formation of multinucleated giant cells in fibroblasts treated with polyethylene glycol (7). More recently, PMA was shown to influence the expression of HSPs in rat embryo fibroblasts (44); and other shock treatments, such as UV irradiation and viral reactivation (45), induced HSPs as well.

Thus, we are continuing to document the expression of the "classical" HSPs (stress proteins) under a variety of conditions. However, similar to the induction of heme oxygenase (42), we are finding that other proteins, e.g., collagenase and stromelysin, can also be classified as heat shock inducible. The ability of heat shock to induce metalloproteinase mRNAs and proteins suggests a pathophysiological role for increases in temperature during chronic inflammatory states.

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