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Characterisation of chondrocyte activation in response to cytokines synthesised by a synovial cell line

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The lapine, synovial cell line, HIG-82, secretes 'chondrocyte activating factors' (CAF) which induce the synthesis of collagenase (EC 3.4.24.7), gelatinase, caseinase and prostaglandin E_2 (PGE_2) by confluent, monolayer cultures of lapine, articular chondrocytes. Partially purified CAF increased the production of PGE_2 by chondrocytes within 3 h; in certain cultures this occurred in as little as 1 h. Increased levels of the three neutral metalloproteinases, in contrast, were only measurable in the conditioned medium after a delay of 9–18 h. After removal of the CAF, the synthesis of PGE_2 reverted to basal levels within 1–4 h, but synthesis of the three proteinases remained high for an additional 4 days. Indomethacin, at concentrations which completely inhibited PGE_2 synthesis, had no effect upon the coordinate induction of collagenase, gelatinase and caseinase. However, cycloheximide, α-amanitin and 5,6-dichlororibosylbenzimidazole (DRB) suppressed induction of these proteinases suggesting that CAF derepressed the genes coding for these enzymes. Once the chondrocytes had been activated by CAF, the inhibitors of transcription had a much weaker effect on the production of the neutral proteinases, indicating that their mRNAs may be relatively stable. In the presence of CAF, inhibition under these conditions was weaker still, possibly due to stabilisation of these mRNA molecules. Experiments with a number of compounds which modulate cellular Ca^{2+}, cAMP or cGMP failed to support a straightforward role for these mediators in the induction of neutral metalloproteinases in chondrocytes. High concentrations of phorbol myristate acetate (PMA) provoked only a slight synthesis of these enzymes.

Introduction

Cytokines from synovium [1–3], polymorphonuclear leucocytes [4,5] and cells of the monocyte-macrophage lineage [6–8] augment the synthesis of PGE_2 and the neutral proteinases, collagenase (EC 3.4.24.7), gelatinase and caseinase by monolayer cultures of articular chondrocytes. This process is known as chondrocyte activation. Such cytokines also promote the autolytic digestion of fragments of living cartilage [9–12], in a response originally ascribed to 'catabolin' activity [13]. Since the co-identity of catabolin and interleukin-1 (IL-1) was established [13], attention has focussed upon the modulation of chondrocyte metabolism by IL-1. Highly purified IL-1, obtained by standard biochemical methods or through recombinant techniques, both activates

Abbreviations: CAF, chondrocyte activating factors; PGE_2, prostaglandin E_2; DRB, 5,6-dichlororibosylbenzimidazole; PMA, phorbol myristate acetate; IL-1, Interleukin-1; HCCH, hexachlorocyclohexane; APMA, aminophenylmercuric acetate; TCA, trichloroacetic acid.

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cultured chondrocytes [13–16] and possesses catabolin activity [13]. It is presently thought to be the key cytokine responsible for modulating chondrocyte metabolism in media contained by the cells mentioned above.

Although explants of synovial tissue appear to secrete IL-1 [2,17], it is not known whether this is the only synovial product involved in chondrocyte activation. Partly because of the limited availability of synovial tissue and the small gravimetric quantities of IL-1 they synthesise, synovial IL-1 has not yet been purified to homogeneity [13]. Furthermore, several different cell types are present in synovium and these vary with the pathophysiology of the joint. As an alternative approach, we have developed an established cell line of lapine synovial origin, designated HIG-82 [18]. These cells, which can be cultured indefinitely and in large amounts, secrete cytokines which activate confluent, monolayer cultures of lapine, articular chondrocytes [3]. Although, we have obtained unpublished evidence that IL-1 is indeed a component of culture media conditioned by HIG-82 cells, its full characterisation is still in progress. Thus, for the time being we are referring to the synovial cytokines only as 'chondrocyte activating factors' (CAF). Although purified IL-1 and a number of other interesting cytokines are now available commercially, we have elected to continue working with partially purified preparations of CAF until their biochemical constitution is better understood. Here, we have characterised the response of articular chondrocytes to the CAF produced by HIG-82 cells.

Materials and Methods

The following materials were purchased from the indicated companies: tissue culture plasticware (Fisher Scientific, Pittsburgh, PA); culture media, sera, antibiotics, trypsin, Gey's balanced salt solution (GIBCO, Grand Island, NY); [^{3}H]acetic anhydride (400 mCi · mmol^{-1}), PGE_2 radioimmunoassay kits (New England Nuclear, Boston, MA); type I collagen from bovine skin (Collagen Corp., Palo Alto, CA); young adult male New Zealand White Rabbits (Green Meadows Rabbitry, Murrysville, PA); all other chemicals (Sigma Chemical Co., St. Louis, MO). Hexachlorocyclohexane (HCCH) was generously donated by Dr. R.E. Basford, University of Pittsburgh School of Medicine.

Cell culture. The HIG-82 cell line is fibroblastic, and was established by spontaneous transformation of a late passage culture of primary lapine synoviocytes. These cells are pseudodiploid and have a doubling time of approx. 24 h [18].

HIG-82 cells at passages 90–130, were cultured in Ham's F-12 medium supplemented with 10% (w/v) fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. When confluent, cultures were detached by treatment with trypsin (0.25%, w/v) for 15–20 min and subcultured at a 1 : 2 or 1 : 4 split ratio.

For chondrocyte cultures, rabbits were killed by intravenous injection of 4 ml Nembutal (50 mg/ml) and articular cartilage was removed from their shoulder and knee joints. Chondrocytes were obtained by sequential digestion with trypsin (0.2%) and collagenase (0.2%) according to the method of Green [19]. The chondrocytes were seeded into 24-well plates at a concentration of $2 \cdot 10^4$ cells per well, with 1.5 ml medium. Cultures became confluent after 8 – 11 days incubation at 37 °C in a 5% CO_2 atmosphere, at which time they were used for activation studies.

Preparation of CAF. Synthesis of CAF was induced by treating confluent monolayers of HIG-82 cells in 75 cm^2 flasks with 0.1 μg/ml PMA [3]. After 3 days further incubation, the conditioned media were recovered and treated for 1 h with 1 mM aminophenylmercuric acetate (APMA). This process activated latent, synovial, neutral proteinases, promoting their interaction with the inhibitor α_2-macroglobulin present in the serum and facilitating their subsequent precipitation. Solid ammonium sulphate was added to 60% saturation. Following overnight storage at 4 °C, the precipitate was recovered by centrifuging and additional ammonium sulphate added to the supernatant to bring it to 95% saturation. After another night at 4 °C, the second precipitate was recovered and redissolved in saline (NaCl, 0.9%, w/v) solution. This solution was then dialysed against a large volumetric excess of saline solution to provide a preparation lacking synovial proteinases, PGE_2 and residual PMA, yet which activated chondro-

cytes. Each batch of CAF was characterised by a dose-response experiment.

Chondrocyte activation assays. Growth medium was removed from confluent, monolayer cultures of articular chondrocytes and the cell sheet washed three times with Gey's balanced salt solution. Neuman-Tytell medium (1 ml) was added to each well in the presence or absence of CAF. A volume of 10–20 μl CAF, containing approx. 200 μg protein, was normally required for maximal activation. The appropriate amounts of the various putative inhibitors or activators were also added to various wells at the concentrations indicated in the results section.

Following 3 days further incubation without refeeding, the conditioned media were recovered and assayed for PGE_2, collagenase, gelatinase and caseinase. The cell sheets were trypsinised and the cells in each well were counted with a haemocytometer.

Neutral collagenase, gelatinase and caseinase activities were determined by radioactive assay with tritiated substrates. Collagen and casein were radioactively labelled by treatment with [^{3}H]acetic anhydride by the method of Gisslow and McBride [20]. Certain batches of collagen were then heated at 60°C for 30 min to form gelatin. The specific activities of collagen and gelatin were then adjusted to 1000 cpm/μg and that of casein was adjusted to 10000 cpm/μg. Collagenase assays were conducted at 35°C with a microfibril assay. Radioactive collagen (25 μl, 75 μg) was placed into a microfuge tube and allowed to gel by incubating at 35°C for 2 h. To the gel was then added 20 μl conditioned medium and 30 μl of reaction buffer (30 mM Tris-HCl, 0.2 M NaCl and 5 mM $CaCl_2$ pH 7.2) with or without APMA (1 mM). The integrity of the collagen was monitored with a pair of trypsin (10 μg) control assays in each run. Following 4 h incubation at 35°C, the reaction was stopped by adding 50 μl EGTA (10 mM final concentration), undigested fibrils were pelleted by centrifuging at $12000 \times g$ for 10 min, and the radioactivity of 50 μl of the supernatant measured by liquid scintillation.

For gelatinase assays, [^{3}H]gelatin (25 μl, 75 μg), 20 μl, conditioned medium and 30 μl reaction buffer with or without APMA were incubated for 4 h at 37°C. The reaction was stopped and undigested gelatin was precipitated by the addition of 75 μl of 40% trichloroacetic acid. The mixture was centrifuged at $12000 \times g$ for 10 min and the radioactivity of a 50 μl aliquot of the supernatant was measured.

Neutral caseinase assays were conducted in the same manner, but with 20 μl casein (30 μg) as substrate and 100 μl of 6% trichloroacetic acid and 50 μl (75 μg) of unlabelled carrier casein added at the end of the reaction. For caseinase assays, the radioactivity of 100 μl of the trichloroacetic acid supernatant was measured.

With each enzyme, 1 unit of enzyme activity degrades 1 μg of substrate per min.

PGE_2 concentrations were determined by a radioimmunoassay kit according to the manufacturer's (New England Nuclear) instructions. The results of a typical experiment are shown in Figs. 1, 3, 4 and 5. In some cases, the presence of the classical vertebrate collagenase was confirmed by electrophoretic separation of the breakdown products as described in Ref. 18.

Analysis of collagen breakdown fragments. The collagenase assay described above provides quantitation of collagen breakdown, but is not strictly diagnostic for collagenase (EC 3.4.24.7). To determine whether chondrocytes were indeed producing the typical vertebrate collagenase, an analysis of the breakdown fragments liberated from collagen by their conditioned media was made. The purpose was to try to identify the characteristic TC^A and TC^B fragments formed as a result of collagenase's cleavage of the collagen molecule at a site approximately 1/4 of the distance from the C terminus. As these fragments denature at 35°C and thus become degraded by less specific proteinases in the conditioned medium, it is necessary to lower the incubation temperature to 25°C to detect them. To this end media were pretreated with 1 mM APMA and added to [^{3}H]collagen (type I) at 25°C. Aliquots were removed at the indicated times and subjected to SDS-polyacrylamide electrophoresis using 7% gels. Radioactive bands were located by fluorography.

Results

CAF rapidly increased PGE_2 synthesis by the chondrocytes. Elevated concentrations of PGE_2

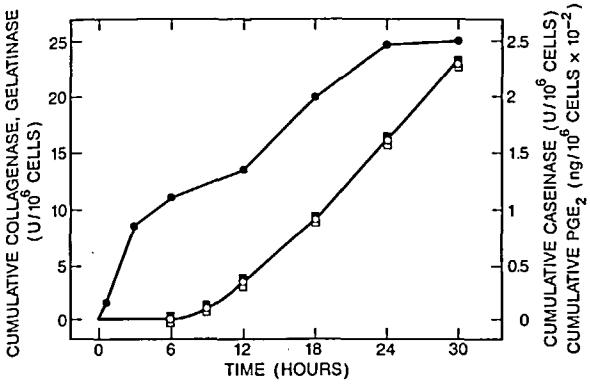

Fig. 1. Time course for the activation of articular chondrocytes by CAF. The CAF was added at zero time. Aliquots of conditioned medium were removed at the indicated times and assayed for PGE_2 (●), collagenase (■), gelatinase (○) and caseinase (□).

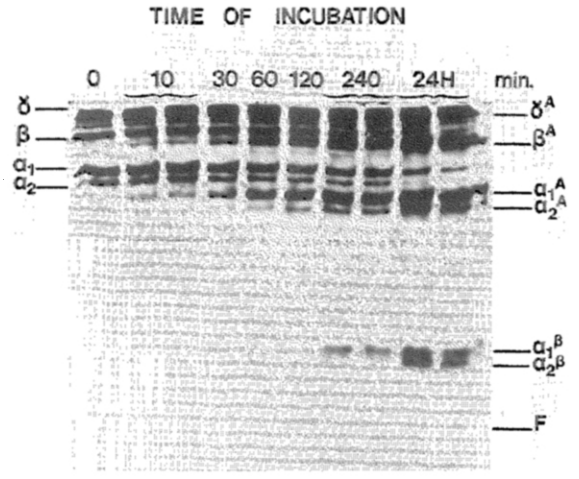

Fig. 2. Analysis of the digestion products formed from collagen through the action of medium conditioned by activated chondrocytes. Media were pretreated with 1 mM APMA and added to [^{3}H]collagen (type I) at 25° C. Aliquots were removed at the indicated times and subjected to SDS-polyacrylamide electrophoresis using 7% gels. Radioactive bands were located by fluorography. This figure shows the characteristic TC^A (marked α_1^A, α_2^A) and TC^B (marked α_1^B, α_2^B) fragments. Alpha chain dimers (β) and trimers (γ) are also degraded into their characteristic products (β^A, γ^A). F marks the position of the tracking dye at the bottom of the gel.

were present in the conditioned medium within 3 h of addition of CAF; in some cultures this could be measured after only 1 h (Fig. 1). Enzyme induction was a much slower process, with collagenase, gelatinase and caseinase being measurable in the conditioned medium only after a protracted lag period. This varied from rabbit to rabbit, but was never shorter than 9 h (Fig. 1) nor longer than 18 h. Moreover, under our experimental conditions, the three neutral metalloproteolytic activities always appeared together. For clarity, only the data for the induction of collagenase will be presented from here on. The collagenolytic activity of medium conditioned by activated chondrocytes was inhibited by EGTA and *O*-phenanthroline (data not shown), while electrophoretic analysis of the digestion products (Fig. 2) clearly identified the characteristic 3/4 (TC^A) and 1/4 (TC^B) fragments which typify the action of vertebrate collagenase.

PGE_2 production by activated chondrocytes rapidly declined following the removal of CAF, reaching basal levels within 1–4 h. However, under these circumstances the synthesis of the three neutral proteinases continued at approx. 75% of the activated level for at least 4 days (Fig. 3). Indomethacin (10^{-7}–10^{-5} M) completely inhibited the synthesis of PGE_2 by chondrocytes in response to CAF, without affecting the induction of collagenase, gelatinase and caseinase (data not shown). Furthermore, direct addition of PGE_2

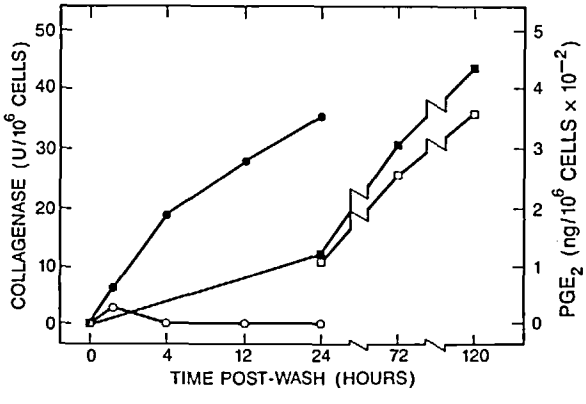

Fig. 3. Reversibility of the induction of PGE_2 and neutral metalloproteinases by CAF. Cultures of lapine chondrocytes were activated with CAF for 24 h. The media were then removed, the cells washed and fresh medium added in the presence (closed symbols) or absence (open symbols) of CAF. Aliquots of conditioned medium were removed at the indicated times and assayed for PGE_2 (circles) or collagenase (squares), gelatinase and caseinase (not shown). All three neutral metalloproteinases acted coordinately.

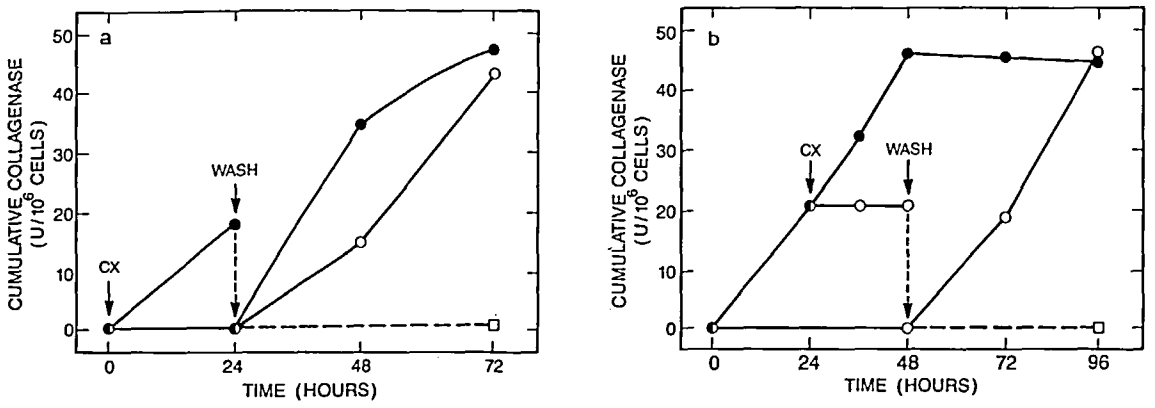

Fig. 4. Reversible inhibition by cycloheximide of the induction and production of neutral metalloproteinases by cycloheximide. (a) Cycloheximide (CX; 5 μg/ml) was added to unactivated chondrocyte cultures at the same time as CAF (○). Positive control cultures were exposed to CAF only (●) and negative controls (□) received neither CAF nor cycloheximide. After 24 h, all cultures were washed and the positive and negative controls were reestablished. At this time, the cycloheximide-containing cultures were washed free of cycloheximide and recultured in fresh medium lacking cycloheximide. Aliquots of conditioned media were assayed for collagenase (as shown), gelatinase and caseinase (not shown) at the indicated times. All these enzymes behaved in a coordinate fashion. (b) Cultures were established in the presence (●, ○) or absence (□) of CAF. At 24 h, cycloheximide (5 μg/ml) was added to certain CAF-containing cultures (○); other CAF-containing cultures (●) were not exposed to it. At 48 h, cycloheximide was removed from the cultures by washing, and the cells transferred to medium lacking cycloheximide, but containing CAF. Aliquots of conditioned medium were withdrawn at the indicated times and assayed for collagenase (as shown), gelatinase and caseinase (not shown). All the enzymes behaved coordinately. Aliquots of conditioned medium were withdrawn daily from all wells and assayed for the neutral metalloproteinases, all three of which behaved in the same manner.

(10^{-5} to 10^{-8} M) to unactivated chondrocytes failed to provoke synthesis of these enzymes (q.v.).

The effects of cycloheximide upon enzyme induction were tested at doses from 0.5 to 50 μg/ml. As prolonged exposure to cycloheximide can be toxic, reversibility experiments were conducted to confirm viability. such experiments identified 5 μg/ml as a dose of cycloheximide which completely but reversibly suppressed both the induction of proteolytic activity (Fig. 4a) and its continued synthesis in activated cells (Fig. 4b).

TABLE I

REVERSIBLE INHIBITION BY DRB OF COLLAGENASE INDUCTION BY CAF

	Collagenase production (U per 10^6 cells per 3 days)			
DRB concn. (μg/ml):	0	5	25	50
DRB present continuously (3 days)	57.3	36.5	10.2	3.3
DRB removed after 48 h treatment; 3 days further incubation	56.5	55.9	52.8	48.9

Having thus demonstrated the need for translation, we next tested inhibitors of transcription. At does from 5 to 100 μg/ml, α-amanitin completely

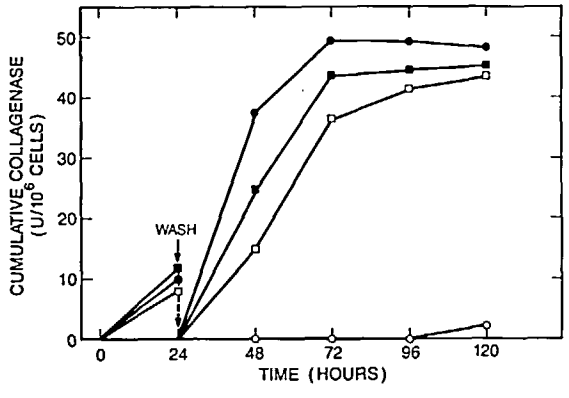

Fig. 5. Effect of DRB on neutral proteinase synthesis by activated chondrocytes. At zero time, CAF was added to all wells apart from negative controls (○). At 24 h, the cultures were washed and these controls were re-established (○). The previously activated cultures were now divided into three groups. One (●), received CAF, as before. Another (□) received DRB (50 μg/ml) only. The third group received both CAF and DRB (■). Samples were withdrawn daily from all wells and assayed for the three neutral metalloproteinases. All acted as shown here for the collagenase data.

suppressed enzyme induction (data not shown). However, as α-amanitin is an irreversible inhibitor, reversibility experiments could not be undertaken to address the issue of toxicity. For this reason, we turned to the reversible inhibitor of transcription, DRB. At concentrations up to 50 μg/ml, DRB inhibited enzyme induction by CAF in a dose-dependent, reversible manner (Table I). In certain experiments, the chondrocytes were first activated with CAF for 24 h, before removing the CAF and adding DRB. Under these conditions, proteinases continued to accumulate in the conditioned medium at approx. 75% of control levels. If the experiment was repeated with CAF continually present, this figure rose to nearly 90% of controls (Fig. 5). Of interest is the observation that the concentrations of PGE_2 (Fig. 1) and the proteases (Figs. 4b and 5) in the medium tended to plateau.

Working on the observation that proteinase synthesis in CAF-treated chondrocytes seemed to involve an 'induction' phase of 9 h or more, which was sensitive to inhibition of transcription, followed by a 'production' phase of several days, which was relatively insensitive to such inhibition, we conducted the following experiment. Cycloheximide (5 μg/ml) was added to the chondrocytes during either the 'induction' phase only or during the 'production' phase only. As expected, cycloheximide rapidly and completely suppressed the continued synthesis of collagenase, gelatinase and caseinase by activated cells (Fig. 4b). Surprisingly, it also reversibly suppressed the accumulation of these enzymes when present only during the 'induction' phase (data not shown), at a time too early for its effects to be explained by inhibition of the translation of the mRNAs coding for these enzymes.

Cellular activation by IL-1 is mediated via cell surface receptors [21,22], for which no second messenger has yet been identified. Preliminary experiments were conducted to screen for possible second messengers involved with the induction of neutral metalloproteinases in chondrocytes. Three groups of reagents were tested for their ability to provoke the synthesis of collagenase, gelatinase and caseinase or to modulate enzyme induction by CAF. In the first group were compounds which modify the cytosolic concentrations of free Ca^{2+};

TABLE II

EFFECTS OF VARIOUS MODULATORS OF PUTATIVE SECOND MESSENGERS UPON THE SYNTHESIS OF NEUTRAL METALLOPROTEINASES BY ARTICULAR CHONDROCYTES

Compound	Maximum enzyme induction (% of CAF ± S.D.)	(*n*)
Ionomycin (10^{-8} to 10^{-4} M)		(4)
HCCH (10^{-5} to $1.5 \cdot 10^{-4}$ M)	0	(1)
8-bromo cAMP (10^{-5} to 10^{-2} M)	2.3 ± 5.7	(6)
8-bromo cGMP (10^{-5} to 10^{-2} M)	3.7 ± 6.1	(7)
PGE_2 (10^{-8} to 10^{-5} M)	0	(11)
NaF (10^{-5} to 10^{-1} M)	0	(1)
PMA (10^{-4} to 1 μg/ml)	13.2 ± 7.3	(12)
Vanadate ($2 \cdot 10^{-7}$ to 10^{-3} M)	0	(9)
Ionomycin + 8 bromocAMP	0	(1)
Ionomycin + 8 bromocGMP	0	(1)

the ionophore ionomycin and HCCH, which liberate Ca^{2+} from intracellular stores in certain cells [23]. Secondly, 8-bromo cAMP, 8-bromo cGMP, NaF and PGE_2 were employed to mimic or to modulate cyclic purine nucleotides. Lastly, PMA was used to activate protein kinase C [24] and vanadate to inhibit dephosphorylation of tyrosine residues [25]. As shown in Table II, none of the substances in the first two categories had any effect whatsoever, either alone or in combination. PMA, but not vanadate, had a weak activating effect at a high dose.

Discussion

Although the activation of chondrocytes by synovial products has been shown before [1–3], the kinetics and mechanism of this activation have not been previously addressed. Furthermore, the CAF used in the present work was derived from a specific synovial cell type, instead of the usual heterocellular fragments of whole tissue.

The rapid elevation in PGE_2 synthesis agrees with the data of Evequoz et al. [8] who exposed lapine chondrocyte cultures to lapine macrophage cytokines. Purified, human IL-1 induces PGE_2 synthesis in lapine chondrocytes within 1–6 h [26]. Chondrocytes thus seem to increase their synthesis of PGE_2 in response to activators quicker than synovial fibroblasts do, where lag periods of 12–24

h [27], 6–12 h [28] and, for HIG-82 cells, around 12 h [29] have been measured.

In contrast to the rapid enhancement of PGE_2 synthesis, we found a marked delay before the neutral proteinases could be detected in increased amounts in the chondrocytes' conditioned media. This finding disagrees with the data of Evequoz et al. [8] who detected no such lag, but agrees with those of Ridge et al. [7] who reported a delay of 8–16 h prior to synthesis of collagenase and a proteoglycan-degrading enzyme in response to macrophage products. Lapine synovial fibroblasts [27] and HIG-82 cells [29] have a similar delay. In view of our data suggesting that proteinase production by chondrocytes in response to CAF requires de novo synthesis via transcription and translation, some sort of lag period should occur. The inability of indomethacin or added PGE_2 to influence the synthesis of the three neutral proteinases confirms that prostaglandin synthesis is not an obligatory step in their induction [3,15,30]. Likewise, the catabolin effect of IL-1 does not depend upon prostaglandin production [31]. Although collagenase induction in primary cultures of synovial cells [32] and HIG-82 cells [29] is, as in chondrocytes, independent of prostaglandin synthesis, this is not so in macrophages [33]. Furthermore, indomethacin does inhibit the synovial production of plasminogen activator in response to IL-1 [34].

Although our unactivated chondrocyte cultures rarely secreted measurable amounts of neutral metalloproteinases into their media, a basal level of proteinase secretion has been observed by other investigators using human, bovine and lapine chondrocytes [35–37]. The basal synthesis of enzymes which degrade proteoglycans seems to be particularly high in culture of bovine chondrocytes [37]. Interestingly, the response of these cells to IL-1 is modest compared to that of lapine and human chondrocytes [37].

That inhibitors of transcription and translation prevent the induction of collagenase, gelatinase and caseinase indicates that in chondrocytes, as in synovial fibroblasts [38,41], macrophages and endothelial cells [41], the production of these enzymes is quantitatively linked to the abundances of their mRNAs. Although our data cannot exclude the suggestion [17] that chondrocytes synthesise procollagenase constitutively, with the collagenase activator proteinase being modulated by CAF, the molecular hybridisation studies of Stephenson et al. [42] indicate a true induction of collagenase mRNA in human chondrocytes. We [43] have obtained similar, preliminary data with lapine chondrocytes. Collectively, these results also counter the possibility that the apparent induction of collagenase actually reflects reduced synthesis of an inhibitor such as TIMP [44].

As the synthesis of the three neutral metalloproteinases by CAF-activated chondrocytes continued at only a modestly reduced rate after removal of CAF or addition of inhibitors of transcription, their mRNA molecules may have relatively long half-lives. In the continued presence of CAF, the effect of the transcriptional inhibitors was even weaker, possibly due to message stabilisation by CAF. If so, chondrocytes would parallel synovial fibroblasts, where activators like PMA extend the half-life of the collagenase message to as long as 36 h [40]. However, although Ridge et al. [7] also found the synthesis of neutral proteinases by chondrocytes remained stable after removal of the activator, others [14] have not done so.

The early effect of cycloheximide reported in this paper is compatible with the findings of Frisch et al. [41] who noted that cycloheximide blocked the appearance of stromelysin and collagenase mRNA in lapine synovial fibroblasts. Our latest data indicate that this is also so for lapine articular chondrocytes [45]. Cycloheximide also inhibits the appearance of thymidine kinase mRNA during the $G_1 \rightarrow S$ transition of 3T3 cells [46].

Our experiments on possible second messengers, failed to identify a clear role for Ca^{2+} or cyclic purine nucleotides in the induction of collagenase, gelatinase and caseinase in chondrocytes. However, inasmuch as it is impossible to prove a negative, we cannot exclude their possible, more cryptic, intervention. Nevertheless, the studies of Carroll [28] weigh against cAMP as a mediator in the stimulation of porcine synovial PGE_2 synthesis by IL-1. However, Ca^{2+} ionophores increase PGE_2 production by lapine chondrocytes [47]. Furthermore, cAMP seems to be involved in the induction of plasminogen activator by IL-1 in synovial fibroblasts [34]. Clearly, the possibility

for separate intracellular mediators for the various pleiotropic effects of IL-1 and CAF exists.

Although PMA is a powerful activator of lapine synovial fibroblasts [27], it elicited only a weak induction of collagenase, gelatinase and caseinase, in our lapine chondrocytes. However, it appears to be a strong inducer in human chondrocytes [42]. We [48] find that PMA acts synergistically with recombinant human IL-1 in activating lapine chondrocytes. These findings suggest that studies of the role of protein phosphorylation in the induction of neutral metalloproteinases may be rewarding. Whatever derepression mechanism is in operation, one of its intriguing features is that induction of collagenase, gelatinase and caseinase is always coordinate.

Acknowledgements

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