Adenylate cyclase of human articular chondrocytes

Responsiveness to prostaglandins and other hormones

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Adenylate cyclase [ATP pyrophosphate lyase (cyclizing), EC 4.6.1.1] was shown to be present in cultured human articular chondrocytes. Optimal conditions of incubation time, protein and substrate concentrations and pH were determined in whole cell lysates. Maximal activity occurred at pH 8.5 with no decrease in activity up to pH 10.0. Adenylate cyclase activity of particulate membrane preparations was enhanced by the addition of crude cytosol preparations. The prostaglandins E_1 , E_2 , $F_{1\alpha}$, $F_{2\alpha}$, D_2 , B_1 , B_2 , A_1 and A_2 , as well as adrenaline and isoprenaline, stimulated adenylate cyclase derived from either adult or foetal chondrocytes. No significant stimulation was observed in the presence of human calcitonin or glucagon. Bovine parathyroid hormone always significantly stimulated the adenylate cyclase derived from foetal chondrocytes, but not from adult chondrocytes. Preincubation of the chondrocytes in culture with indomethacin and with or without supernatant medium from cultured mononuclear cells increased the responsiveness of the adenylate cyclase to prostaglandin E_1 .

Little work has been done previously on the adenylate cyclase of chondrocytes of any species. In the studies which have been reported, the effect of prostaglandins has been variable. Tell *et al.* (1973), working with a homogenate of chick cartilage, observed a significant stimulatory response to bovine parathyroid hormone but not to any of the prostaglandins. Smith *et al.* (1976) also found that bovine parathyroid hormone and porcine calcitonin significantly stimulated adenylate cyclase of rat chondrocytes and that there was a small response to prostaglandins E_1 and E_2 . Recent work by Meats *et al.* (1980a) has shown that human articular chondrocytes *in vitro* may be stimulated by factors produced by human mononuclear cells and by synovial tissue to produce large amounts of prostaglandin E. This has suggested that prostaglandin E might play a role in cartilage metabolism. The aim of our study was to investigate the responsiveness of the adenylate cyclase enzyme [EC 4.6.1.1., ATP pyrophosphate-lyase (cyclizing)] of cultured human articular chondrocytes to prostaglandins and other hormones. In addition we have studied the effects of

the cell cytosolic fraction and of endogenously produced prostaglandins on adenylate cyclase activity in these cells.

Materials and methods

Chemicals

Eagle's minimal essential medium (Earle's salts), foetal calf serum, and Dulbecco's Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline were from Gibco Europe, Paisley PA3 4EP, Scotland, U.K.

Enzymes used for isolation of the chondrocytes were: 0.25% trypsin from Gibco and clostridial collagenase (type I) and bovine testicular hyaluronidase (type V) from Sigma.

Amersham International supplied [α - ^{32}P]ATP (0.5–30 Ci/mmol) and cyclic [8- 3H]AMP (20–30 Ci/mmol). Cyclic AMP (crystallized free acid), ATP (disodium salt), pyruvate kinase (sp. activity approx. 200 EC units/mg) and phosphoenolpyruvate (tricyclohexylammonium salt) were from Sigma. Bovine serum albumin was supplied by Armour Pharmaceuticals, Eastbourne, Sussex, U.K. All other chemicals were of AnalaR grade from BDH.

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Hormones

Prostaglandins were a gift from Dr. John Pike of the Upjohn Company, Kalamazoo, MI, U.S.A. Bovine parathyroid hormone (2500 i.u. per mg) was provided by the National Institute of Biological Standards, Holly Hill, Hampstead, London NW3 6RB, U.K. Synthetic human calcitonin was a gift from Ciba-Geigy, Basel, Switzerland. Glucagon (1000 units/ml) was supplied by Eli Lilly and Co.; L-adrenaline (injection B.P.) by Antigen, Roscrea, Ireland; L-isoprenaline (hydrochloride B.P.) by Pharmax, Bexley, Kent, U.K.; anti-diuretic hormone as vasopressin injection B.P. (20 international pressor units/ml) by Parke, Davis and Co., Pontypool, Gwent, U.K.; L-noradrenaline by Winthrop Laboratories, Surbiton-on-Thames, Surrey, U.K.

Chondrocyte culture

Adult human articular cartilage was obtained from the knee joints of lower limbs amputated due to ischaemia and also from femoral heads following fracture of the femoral neck. Foetal articular cartilage was excised from the femoral heads and femoral condyles after early neonatal death. Only cartilage which appeared macroscopically normal was used.

Chondrocytes were isolated by the method of Srivastava *et al.* (1974) with modifications as described by Meats *et al.* (1980b) and cultured in Eagle's minimal essential medium with Earle's salts supplemented with 10% (v/v) foetal calf serum and penicillin (100 units/ml) and streptomycin (100 µg/ml) at 37°C in an atmosphere of CO₂/air (1:19). Experiments were performed on chondrocytes which had been subcultured only once and had reached confluence.

Preparation of cells for assay of adenylate cyclase activity

Culture medium was poured from monolayers of confluent chondrocytes and the cell layer was rinsed with 10 mM-Tris/HCl buffer (pH 7.4 at 4°C) containing 0.25 M-sucrose and 1 mM-EDTA. The cells were scraped from the dish and resuspended in buffer to give the required protein concentration (500–600 µg/ml). A homogenate was prepared by rapidly freezing the cell suspension using a mixture of solid CO₂ and ethanol followed by thawing at 37°C. Whole cell homogenates were used throughout the experiments described unless otherwise indicated.

Particulate preparations of the adenylate cyclase were made as follows: homogenates were centrifuged for 10 min at 5500 g (r_{av} , 10.8 cm) at 4°C and the resultant supernatant (crude cytosol preparation) was retained and the pellet suspended in the buffer described above.

Protein concentrations of the whole cell homo-

genates and particulate preparations were measured by the method of Hartree (1972) using bovine serum albumin as a standard.

Adenylate cyclase assay

Adenylate cyclase activity was measured by the conversion of [α -³²P]ATP to cyclic [³²P]3',5'-AMP as described by Hunt *et al.* (1976). Reaction mixtures (100 µl) contained 25 mM-Tris/HCl, 1 mM-[α -³²P]ATP (20–200 d.p.m./pmol), 1 mM-cyclic 3',5'-AMP, 2.5 mM-phosphoenolpyruvate, 1.5 EC units of pyruvate kinase, 130 µg of bovine serum albumin/ml, 30 mM-KCl and 4.5 mM-MgSO₄. Incubations at 37°C were started by the addition of the relevant whole cell homogenate or particulate enzyme preparation and terminated by the addition of 100 µl of 40 mM-ATP and heating at 95°C for 2 min.

The cyclic [³²P]AMP generated in the assay was isolated by the method of Salomon *et al.* (1974).

Adenylate cyclase activity was assayed in triplicate. Results are expressed as means $\pm$ S.E.M. of triplicate determinations. Statistical analyses were carried out by an analysis of variance, the variances being shown to be homogeneous.

Prostaglandin assay

Concentrations of prostaglandin E were determined in unextracted culture media by radioimmunoassay (Levine *et al.*, 1971), utilizing dextran-coated charcoal to separate bound from free [³H]prostaglandin E₂ tracer (Kahn *et al.*, 1974). Antiserum to prostaglandin E was generously provided by Dr. Lawrence Levine, Brandeis University, Waltham, MA, U.S.A., and had similar specificities toward prostaglandins E₂, E₁ and B₂. We have shown previously that the major prostaglandins produced by human chondrocytes are of the E type (Meats *et al.*, 1980b). The concentrations of prostaglandin B are low relative to prostaglandin E and are derived from prostaglandin E under these conditions of culture. Therefore, results were calculated as equivalents of prostaglandin E.

Mononuclear cell culture

Human blood mononuclear cells were cultured according to the method of Dayer *et al.* (1977). Heparinized peripheral venous blood was drawn from healthy volunteers. The mononuclear cells were isolated on a Ficoll-Hypaque gradient centrifuged at 400 g (r_{av} , 25.4 cm) for 40 min at 15°C and cultured in minimal essential medium supplemented with 10% foetal calf serum and antibiotics at a concentration of 4×10^6 cells/ml. After incubation at 37°C for 72 h, the medium was centrifuged at 3000 g (r_{av} , 25.4 cm) at 15°C for 10 min and stored at -20°C. This crude supernatant medium was used as a source of the mononuclear cell factor. The results

shown are of individual experiments representative of the data obtained on a number of occasions.

Results

Responsiveness of adenylate cyclase in human articular chondrocytes

Investigation of assay conditions indicated that the activity of adenylate cyclase from human articular chondrocytes increased linearly with time for up to 14 min at 37°C for basal and prostaglandin E_1 , adrenaline- and NaF-stimulated enzyme activity. Adenylate cyclase activity also increased linearly with the amount of added protein up to 800 $\mu\text{g}/\text{ml}$. An incubation time of 14 min and a protein concentration of 500–800 $\mu\text{g}/\text{ml}$ was used for all subsequent assays. Enzyme activity was maximal with 1 mM-ATP and 3–10 mM- Mg^{2+} . In the final assay 1 mM-ATP and 6 mM- Mg^{2+} were used. For both adult and foetal chondrocytes, enzyme activity (both basal and that stimulated by prostaglandin E_1 , adrenaline and NaF) was maximal at pH 8.5 and there was no inhibition of enzyme activity even at pH 10.0, the highest pH tested. All assays were subsequently performed at pH 8.5.

A survey of the hormonal responsiveness of adenylate cyclase from human adult articular chondrocytes showed that the enzyme was significantly stimulated by 5 μM -adrenaline, 4.7 μM -iso-

prenaline and by various prostaglandins at 140 μM (see Table 1). The greatest response was seen with the prostaglandins E_1 and E_2 .

Noradrenaline, vasopressin, human calcitonin and glucagon failed to stimulate adenylate cyclase activity at the concentrations used, while in four experiments 2 μM -parathyroid hormone caused a slight stimulation which was not significant. The responsiveness of adenylate cyclase from foetal chondrocytes followed a similar profile with the exception that a significant response to 2 μM -parathyroid hormone was always present (in four experiments). These results are typical of those found on several occasions.

Fig. 1 shows the dose-response curves to prostaglandin E_1 , adrenaline and parathyroid hormone of adenylate cyclase derived from foetal chondrocytes. The lowest concentration of prostaglandin E_1 which significantly stimulated adenylate cyclase activity was 200 nM, and maximal stimulation occurred with 140 μM -prostaglandin E_1 . Parathyroid hormone at 1 and 5 μM stimulated cyclase activity significantly, as did adrenaline at 13.6 and 27.3 μM .

Effect of cytosol

The effect of the cell cytosolic fraction on adenylate cyclase activity was investigated in chondrocytes by comparing the activity of a whole cell lysate with that of a particulate membrane

Table 1. *Survey of hormonal responsiveness of adenylate cyclase from cultured human articular chondrocytes*
Whole cell homogenates were prepared from cultured chondrocytes derived from either adult or foetal cartilage as described in the text. Results are expressed as means $\pm$ S.E.M. of triplicate determinations. Significant stimulation of activity over basal values was estimated by an analysis of variance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Additions	Adenylate cyclase activity (pmol of cyclic AMP/min per mg of cell protein)	
	Adult	Foetal
Basal	35.7 $\pm$ 2.3	53.3 $\pm$ 1.8
140 μM -Prostaglandin E_1	167.2 $\pm$ 4.5***	231.3 $\pm$ 16.0***
150 μM -Prostaglandin E_2	120.1 $\pm$ 3.0***	185.0 $\pm$ 4.3***
140 μM -Prostaglandin F_1	65.3 $\pm$ 12.4*	84.0 $\pm$ 3.0*
140 μM -Prostaglandin F_2	77.6 $\pm$ 2.0**	148.2 $\pm$ 11.8***
150 μM -Prostaglandin D_2	93.1 $\pm$ 5.5***	215.2 $\pm$ 31.0***
150 μM -Prostaglandin B_1	110.7 $\pm$ 19.2***	170.7 $\pm$ 6.8***
150 μM -Prostaglandin B_2	59.5 $\pm$ 8.9	98.0 $\pm$ 7.2**
150 μM -Prostaglandin A_1	118.7 $\pm$ 24.0***	166.8 $\pm$ 6.2***
150 μM -Prostaglandin A_2	82.0 $\pm$ 19.0***	108.3 $\pm$ 4.8***
5.0 μM -Adrenaline	87.1 $\pm$ 2.0***	119.4 $\pm$ 1.3***
4.7 μM -Isoprenaline	82.7 $\pm$ 6.1***	125.5 $\pm$ 7.7***
6.0 μM -Noradrenaline	30.5 $\pm$ 7.1	77.0 $\pm$ 0.8
Vasopressin (200 units/ml)	23.8 $\pm$ 4.3	51.6 $\pm$ 5.3
2.0 μM -Parathyroid hormone (assuming M_r 9500)	50.0 $\pm$ 9.0	86.7 $\pm$ 4.1*
11.0 μM -Calcitonin (human) (assuming M_r 4500)	21.8 $\pm$ 3.8	50.7 $\pm$ 5.8
1.0 μM -Glucagon	38.5 $\pm$ 4.9	48.5 $\pm$ 2.6

Fig. 1. Stimulation of adenylate cyclase derived from cultured human chondrocytes by prostaglandin E_1 , adrenaline and parathyroid hormone

Whole cell homogenates of confluent chondrocytes were prepared and adenylate cyclase assays were performed as described in the Materials and methods section with an incubation time of 14 min at 37°C. Each point represents the mean $\pm$ S.E.M. of triplicate incubations. $\circ$, Basal activity; $\blacksquare$, parathyroid hormone; $\bullet$, prostaglandin E_1 ; $\blacktriangle$, adrenaline. Results significantly different from the basal value are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2. Enhancement by cytosol of hormone-stimulated activity of adenylate cyclase from human articular chondrocytes

Whole cell homogenates of chondrocytes derived from human adult articular cartilage were prepared and divided into two portions. A particulate membrane preparation was prepared by centrifuging the homogenate, removing the supernatant (crude cytosol, containing 420 μ g of protein/ml) and resuspending the pellet in the homogenization buffer, as described in the Materials and methods section. The remaining fraction was similarly centrifuged but resuspended in the supernatant. Adenylate cyclase activity was determined in each preparation. There was no detectable adenylate cyclase activity in the supernatant. The results are given as means $\pm$ S.E.M. of triplicate incubations. Values for adenylate cyclase activity in the particulate membrane preparation (without supernatant) which are significantly different from those in the whole cell homogenate (with supernatant) are indicated: * $P < 0.05$; *** $P < 0.001$.

Additions	Adenylate cyclase activity (pmol of cyclic AMP/min per mg of particulate protein)	
	With supernatant	Without supernatant
Basal	109.1 $\pm$ 8.1	14.0 $\pm$ 0.5*
14 μ M-Prostaglandin E_1	291.1 $\pm$ 51.6	99.0 $\pm$ 3.8***
140 μ M-Prostaglandin E_1	341.9 $\pm$ 39.6	81.0 $\pm$ 2.1***
2.7 μ M-Adrenaline	74.0 $\pm$ 12.1	73.9 $\pm$ 11.6*
27 μ M-Adrenaline	136.3 $\pm$ 27.0	40.5 $\pm$ 8.4*
10 mM-NaF	566.2 $\pm$ 32.2	507.7 $\pm$ 9.2

preparation. The results are shown in Table 2. Basal and prostaglandin E_1 - and adrenaline-stimulated activities were reduced 66–87% in the particulate membrane preparation (i.e. minus cytosol). NaF-stimulated activity was unaffected, however. The crude cytosol preparation lacked any detectable adenylate cyclase activity.

Enhancement of adenylate cyclase activity after preincubation of chondrocytes with indomethacin

Human articular chondrocytes in monolayer cultures produced steady but low levels of prostaglandin E (15 ng/mg of protein per 24 h). To

investigate whether this endogenous production of prostaglandin E might have an effect on subsequent responsiveness to prostaglandin E_1 in the cyclase assay, we incubated the cells with 1.4 μ M-indomethacin for 2 h before preparing the whole cell homogenate. The concentration of prostaglandin E in the culture medium after the 2 h pre-incubation with indomethacin was 0.008 μ M compared with 0.048 μ M in the control. The results are shown in Fig. 2 as fold-stimulation of adenylate cyclase activity over basal values. Preincubation with indomethacin significantly enhanced the responsiveness of adenylate cyclase to concentrations of prostaglandin

$E_1 \geq 10^{-6}$ M. To investigate further this effect we incubated chondrocytes for 2 h with indomethacin in the absence or presence of prostaglandin E_1 at two

concentrations before determining adenylate cyclase activity. The concentration of prostaglandin E in the control culture medium was $0.03 \mu\text{M}$ and levels in the indomethacin-treated cultures were undetectable. The results in Table 3 show that even low concentrations ($0.06 \mu\text{M}$) of prostaglandin E_1 are able to desensitize the cells to further stimulation by the hormone and pre-incubation with the high concentration reduced cyclase responsiveness markedly.

Effect of mononuclear cell factor

In order to investigate mechanisms of cellular interaction which might determine sensitivity of adenylate cyclase in chondrocytes, we preincubated our cells for 23 h with mononuclear cell factor in the presence or absence of indomethacin (Table 4). The factor markedly stimulated the production of prostaglandin E by the chondrocytes (794 ng/mg of protein compared with 35 ng/mg of protein in the control) and this increase was inhibited by the addition of $1.4 \mu\text{M}$ -indomethacin (10 ng of prostaglandin E/mg of protein). Preincubation with mononuclear cell factor reduced prostaglandin E_1 -stimulated activity of adenylate cyclase. However, when the cells were preincubated with indomethacin alone or with mononuclear cell factor plus indomethacin, prostaglandin E_1 -stimulated activity was restored to levels greater than those in preparations from control cultures. Adrenaline- and parathyroid hormone-stimulated activities were increased significantly only when the cells were preincubated with both mononuclear cell factor and indomethacin, although basal activity was also increased in this preparation.

Fig. 2. Effect of preincubation of chondrocytes with indomethacin on adenylate cyclase activity

Confluent adult human articular chondrocytes were preincubated with $1.4 \mu\text{M}$ -indomethacin for 2 h, after which homogenates were prepared. Prostaglandin E_1 -stimulated adenylate cyclase activities were compared in homogenates from cells cultured in the absence (●) or the presence (○) of indomethacin. Results are expressed as fold-stimulation over basal activity (-----) and represent the means $\pm$ S.E.M. of triplicate incubations. Values from indomethacin-treated cells significantly different from those from untreated cells are indicated: * $P < 0.05$, ** $P < 0.01$.

Table 3. Prostaglandin E_1 -stimulated adenylate cyclase activity in chondrocyte homogenates after preincubation in culture with indomethacin and prostaglandin E_1

Confluent adult chondrocytes were incubated with $1.4 \mu\text{M}$ -indomethacin and prostaglandin E_1 , as indicated, for 2 h, then the culture medium was removed and the cell layer washed as described in the Materials and methods section. Whole cell homogenates were prepared from each incubation and adenylate cyclase activity determined. The values are the means $\pm$ S.E.M. of three determinations. The figures in parentheses indicate the fold-stimulation over basal activity. Values significantly different from control values are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Adenylate cyclase activity (pmol of cyclic AMP/min per mg of cell protein)						
Preincubation conditions	Addition ...	Basal	Prostaglandin E ₁			NaF (10 mM)
			0.028 μM	0.28 μM	28 μM	
Control (no addition)		75.1 ± 2.14	104.1 ± 1.0 (1.4)	281.8 ± 32.2 (3.8)	596.4 ± 24.9 (7.6)	758.1 ± 2.5 (10.1)
Indomethacin (1.4 μM)		63.6 ± 4.1	124.3 ± 5.2 (2.0)	289.4 ± 2.4 (4.9)	737.5 ± 26.4*** (11.6)	807.4 ± 7.2* (12.7)
Indomethacin and prostaglandin E ₁ (0.06 μM)		53.9 ± 2.7	93.7 ± 3.1 (1.7)	232.4 ± 15.4* (4.2)	555.9 ± 11.3 (9.9)	718.8 ± 28.0 (12.9)
Indomethacin and prostaglandin E ₁ (11.3 μM)		60.4 ± 3.4	71.4 ± 2.5 (1.2)	141.7 ± 2.6*** (2.4)	449.4 ± 23.1*** (7.4)	664.1 ± 60.4*** (11.0)

Table 4. *Effect of preincubation of chondrocytes with mononuclear cell factor on the activity of adenylate cyclase*
 Confluent adult articular chondrocytes were preincubated with 4% (v/v) mononuclear cell factor, 1.4 μ M-indomethacin or both, as indicated for 23 h, then the culture medium was removed and the cell layer washed as described in the Materials and methods section. Whole cell homogenates were prepared from each incubation. The values are the means $\pm$ S.E.M. of three determinations. The figures in parentheses indicate the fold-stimulation over basal activity. Values significantly different from control values are indicated: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

		Adenylate cyclase activity (pmol of cyclic AMP/min per mg of cell protein)				
Preincubation conditions	Addition ...	Basal	Prostaglandin E ₁ (5.6 μ M)	Adrenaline (27 μ M)	Parathyroid hormone (1.3 μ M)	NaF (10 mM)
Control (no addition)		83.4 $\pm$ 3.7	288.0 $\pm$ 8.6 (3.5)	164.9 $\pm$ 5.2 (2.0)	122.0 $\pm$ 6.6 (1.5)	479.9 $\pm$ 3.9 (5.8)
Indomethacin		57.3 $\pm$ 2.6***	380.2 $\pm$ 8.5*** (6.6)	174.8 $\pm$ 5.0* (3.1)	105.9 $\pm$ 5.9 (1.9)	495.0 $\pm$ 21.4 (8.0)
Indomethacin and mononuclear cell factor		132.5 $\pm$ 1.4***	470.2 $\pm$ 6.4*** (3.9)	252.7 $\pm$ 7.0*** (1.9)	154.7 $\pm$ 2.5** (1.2)	446.6 $\pm$ 21.4** (3.4)
Mononuclear cell factor		124.8 $\pm$ 17.3**	261.9 $\pm$ 13.8* (2.1)	182.4 $\pm$ 3.1* (1.5)	131.3 $\pm$ 5.7 (1.1)	482.5 $\pm$ 5.2 (3.9)

Discussion

We have characterized the adenylate cyclase derived from cultured human chondrocytes and investigated the responsiveness of this enzyme to a range of hormones. Although a significant stimulation occurred in response to a variety of hormones, the responses were very low compared with those of adenylate cyclases of other cell types and tissues. This is typical of an adenylate cyclase which is losing its responsiveness, possibly due to the duration of time in culture or to maturation of cells *in vivo*. For example, rat reticulocytes have been shown to lose their responsiveness to β -adrenergic catecholamines during maturation (Bilezikian, 1978). The human foetal chondrocyte enzyme always responded to parathyroid hormone, whereas the chondrocytes from adult cartilage never gave a significant response to this hormone, indicating that this response may be lost during the maturation of chondrocytes. Smith *et al.* (1976) found that adenylate cyclase in rat growth cartilage responded well to bovine parathyroid hormone and porcine calcitonin, E-type prostaglandins, glucagon and adrenaline. This may indicate that only chondrocytes in growth cartilage are able to respond to parathyroid hormone. In the case of the other hormones which we tested, the adenylate cyclase from adult and foetal chondrocytes responded similarly. It is surprising that there was any response to adrenaline at all, since no physiological role for this hormone has been ascribed in cartilage. Although both the foetal and adult adenylate cyclases responded similarly to all of the prostaglandins tested, the prostaglandins of the E-type were the most potent stimulators of all of the hormones tested. The failure of the chondrocyte adenylate cyclase to lose activity at high pH suggests that this

may be an important factor governing the degree of adenylate cyclase activity in chondrocytes. When Pita & Howell (1978) examined aspirates of interstitial fluid from rat epiphyseal cartilage, they found the pH to be more alkaline than expected, i.e. pH 7.6.

Previous work has shown that adenylate cyclase activity is greatly reduced in the absence of the cell cytosol fraction (Mac Neil *et al.*, 1980) and we have confirmed that this also occurs in preparations of human chondrocytes. Although in the present study unfractionated and non-dialysed cytosol was used, work by Crawford *et al.* (1980) has shown that this enhancement of adenylate cyclase activity by the cell cytosol is due to a protein of apparent M_r of 20000–30000 and cannot be explained simply by the presence of endogenous GTP. The presence of this cytosol factor has been demonstrated in many cell types, including freshly isolated human platelets, human bronchial carcinoma (BEN) cells and various animal tissues (Mac Neil *et al.*, 1980). The loss of hormone responsiveness during maturation of rat reticulocytes has been found to be associated with diminished levels of cytosol factor (Shane & Bilezikian, 1981).

The responsiveness of the chondrocyte adenylate cyclase to prostaglandins is particularly interesting in view of the postulated role of these hormones in acute and chronic inflammation (Bonta & Parnham, 1978), for example, in rheumatoid arthritis. Our results show that concentrations of prostaglandin E which have been measured in rheumatoid synovial fluid (Robinson & Levine, 1974) are able to stimulate chondrocyte adenylate cyclase. This may be important for several inter-related reasons. Other work on cartilage *in vitro* has shown that prostaglandin E₁ and E₂ can inhibit glycosaminoglycan synthesis (Malemud & Sokoloff, 1977) and another study (Fulkerson *et al.*, 1979) demonstrated that

E-type prostaglandins induced degradation of proteoglycans. Prostaglandin E may play a modulatory role in the production of collagenase by synovial cells (Dayer & Krane, 1978), and similar mechanisms may operate in chondrocytes contributing to control of cartilage metabolism. It seems possible, therefore, that variations in concentrations of prostaglandins may play a role in cartilage matrix turnover, which becomes disordered in arthritis. In the continued presence of high concentrations of prostaglandins, as during stimulation by mononuclear cell factor, chondrocytes might become desensitized or refractory to further stimulation, and hence modulation, by this mediator. The phenomenon of 'desensitization', or 'down-regulation', of adenylate cyclase is well recognized in many systems. Clark & Butcher (1979) showed that preincubation of monolayer fibroblasts with $5.7\mu\text{M}$ -prostaglandin E_1 or $10\mu\text{M}$ -adrenaline desensitized adenylate cyclase to further stimulation by the respective hormone. The half-life for this change was as short as 2–3 min and reversal occurred with a half-life of 4–6 min after the hormone was removed. Our results have shown that even the low concentrations of prostaglandin E_1 (approx. 50 nM) measured in chondrocyte culture medium are able to give rise to at least partial desensitization to further stimulation by exogenous prostaglandin.

Dayer *et al.* (1977) have shown that production of prostaglandin E by rheumatoid synovial cells is stimulated by a factor derived from cultured human blood mononuclear cells. This factor, designated mononuclear cell factor, may have an important role in the pathogenesis of joint destruction in rheumatoid arthritis. We have reported that a similar factor stimulates production of prostaglandin E and plasminogen activator by human articular chondrocytes (Meats *et al.*, 1980a). Furthermore, Jasini & Dingle (1981) have reported that human mononuclear cell factors stimulate chondrocyte-mediated breakdown of pig or bovine cartilage. These effects are analogous to those of a factor, 'catabolin', derived from synovial tissue which enhances chondrocyte-mediated cartilage breakdown (Fell & Jubb, 1977; Dingle *et al.*, 1979).

Dayer *et al.* (1979a) have suggested that the mononuclear cell factor may play a role in determining the sensitivity of synovial cells to exogenous prostaglandin E_2 , since it potentiates the prostaglandin-stimulated increase in cyclic AMP levels in rheumatoid synovial cells *in vitro* (Dayer *et al.*, 1979a). In the absence of indomethacin, preincubation of our chondrocytes with the mononuclear cell factor resulted in a marked increase in production of prostaglandin E, and the augmented sensitivity to exogenous prostaglandin of adenylate cyclase from cells preincubated with indomethacin alone was lost, perhaps due to desensitization.

Preincubation of the cells with the factor plus indomethacin appeared to enhance prostaglandin E_1 -stimulated adenylate cyclase activity in chondrocyte lysates above that in lysates from cells preincubated with either indomethacin or factor alone. Thus, the mononuclear cell factor appears to be capable of increasing directly hormonal responsiveness independently from its indirect effects on the production of prostaglandin E. Our results are similar to those of Dayer *et al.* (1979a), who observed an increase in responsiveness to prostaglandin E_2 in synovial cells which had been preincubated with mononuclear cell factor plus indomethacin greater than that in cells preincubated with indomethacin alone. They have suggested that mononuclear cell factor may sensitize or 'up-regulate' the cells to prostaglandin E_2 , possibly by increasing the affinity or number of prostaglandin E_2 binding sites (Dayer *et al.*, 1979b). The increased sensitivity to stimulation by prostaglandin E of chondrocytes treated with indomethacin may provide an explanation for the failure of nonsteroidal anti-inflammatory drugs to block the progressive destruction occurring during inflammatory joint disease.

Since human chondrocytes respond to prostaglandin E_1 , a modulatory role for prostaglandins via alterations in adenylate cyclase responsiveness and changes in cyclic AMP levels may be suggested. Such mechanisms may function during normal control of cartilage metabolism, which may become disrupted in the presence of stimulatory factors and/or drugs.

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