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Identification of a novel inflammatory stimulant of chondrocytes

Early events in cell activation by bradykinin receptors on pig articular chondrocytes

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The inflammatory peptide bradykinin stimulated a rapid and transient increase in cytoplasmic [Ca^{2+}] in primary pig chondrocytes, as measured by the fluorescent indicator dye Fura-2. This increase occurred in the absence of extracellular Ca^{2+}, indicating a mobilization from intracellular stores. The elevation in intracellular [Ca^{2+}] was mediated by authentic bradykinin receptors, since it was blocked by the specific bradykinin antagonist [β-(2-thienyl)-L-$Ala^{5,8}$,D-Phe^{7}]bradykinin. Activation of chondrocytes by bradykinin induced a concentration-dependent [ED_{50} (dose for half-maximal response) ~40 nM] accumulation of inositol monophosphate in the presence of LiCl and a concentration-dependent increase in production of prostaglandin E_2. The generation of the secondary mediator prostaglandin E_2 was a biologically relevant output response induced by bradykinin, but chondrocyte responses, such as the rate of entry into DNA synthesis, the rate and pattern of new protein synthesis and the rate of synthesis and resorption of cartilage proteoglycan, were unaltered by bradykinin treatment. Chondrocytes were also shown to be activated by two pharmacological mediators of cytosolic [Ca^{2+}] elevation, i.e. the ionophore A23187 and thapsigargin, which both produced alterations in protein synthesis which were mimicked by bradykinin. Thus Ca^{2+}-sensitive pathways exist which are not functionally responsive to a Ca^{2+}-mobilizing and inositol phosphate-generating hormone, potentially indicating other routes of regulation. These results call attention to bradykinin and related peptides as another class of inflammatory mediators which may regulate physiological and pathological chondrocyte metabolism.

INTRODUCTION

Articular chondrocytes are specialized cells whose main function is to maintain a healthy cartilage matrix, capable of withstanding compressive forces during joint movement. Inflammatory joint diseases, such as rheumatoid arthritis, are characterized by destruction of articular tissues. One class of inflammatory mediators, cytokines such as interleukin 1, is known to be capable of mediating cartilage loss via specific chondrocyte surface receptors (Bird & Saklatvala, 1986), and interleukin-1-like activity has been detected in human joint effusions (Wood *et al.*, 1983). Histamine receptors have also been identified on articular chondrocytes (Taylor *et al.*, 1985), and stimulation with histamine causes increased eicosanoid production (Taylor *et al.*, 1986), but no direct stimulation of cartilage breakdown.

In order to understand fully the biological roles of cartilage-derived cells and the mechanisms of joint destruction during inflammation, it is important to identify the pattern of functional receptors expressed in chondrocytes. A number of inflammatory mediators are known to mobilize intracellular Ca^{2+} via inositol-lipid hydrolysis (Irvine, 1988). These mediators include histamine, acting via H1 receptors (Hepler *et al.*, 1987), neurogenic activating peptides such as the tachykinins (Hanley *et al.*, 1980), platelet-activating factor (Lapetina, 1982), and kinins such as bradykinin (Jackson *et al.*, 1987; Tilly *et al.*, 1987). Here we have used the fluorescent $[Ca^{2+}]_i$ indicator Fura-2 to identify the presence of functional Ca^{2+}-mobilizing receptors on pig chondrocytes. The results suggest that, among known receptors of this class, only bradykinin receptors are expressed on the chondrocyte population. Moreover, bradykinin, a hormonal mediator of intracellular Ca^{2+} discharge, has been compared with pharmacological agents, the inflammatory tumour-promoting plant product thapsigargin, and the Ca^{2+} ionophore A23187, to address the role of $[Ca^{2+}]_i$ elevation in the regulation of chondrocyte function.

MATERIALS AND METHODS

Cell culture

Cartilage was dissected from the metacarpophalangeal joints of pigs aged approx. 25 weeks, taking care to exclude the underlying bone marrow. The dissected pieces of pure cartilage were chopped, washed several times in PBS and incubated overnight in serum-free DMEM (Gibco) containing 1 mg of collagenase (Worthington Enzymes)/ml and 50 μg of gentamycin (Sigma)/ml. Chondrocytes were pelleted by centrifugation at 100 *g* for 5 min. The pellet was washed by resuspension in fresh DMEM, and cells were plated at a density of 1×10^6/well in 24-well plates and 3×10^6/dish in 35 mm-diam. dishes in DMEM containing 5% (v/v) FCS and 5 μg of gentamycin/ml. These high-density

Abbreviations used: $[Ca^{2+}]_i$, cytoplasmic free calcium concentration; PBS, phosphate-buffered saline; HBS, Hanks balanced salts solution; DMEM, Dulbecco's minimal essential medium; FCS, foetal-calf serum; Thi, β-(2-thienyl)-L-alanine; PGE_2, prostaglandin E_2; ED_{50}, dose for half-maximal response.

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primary cultures, consisting exclusively of chondrocytes, were used for experimentation 48–72 h after plating. For fluorescence measurements, cells were plated into 35 mm dishes containing sterile 11 mm × 22 mm glass coverslips which had been pretreated overnight with 5 μg of poly-L-lysine (Sigma)/ml to aid cell attachment.

Measurement of $[Ca^{2+}]_i$

Coverslips with attached chondrocytes were incubated in modified HBS (1 mM-$CaCl_2$, 5.4 mM-KCl, 0.5 mM-$MgCl_2$, 0.2 mM-$MgSO_4$, 137 mM-NaCl, 4.2 mM-$NaHCO_3$, 0.4 mM-NaH_2PO_4, 44 mM-glucose, 20 mM-Hepes, pH 7.4) containing 2 μM-Fura-2/AM (Molecular Probes Inc.) for 45 min at room temperatue. Dye loading at room temperature rather than 37 °C decreased background fluorescence. At the end of the incubation with Fura-2, cells were washed in HBS and placed across the diagonal of a 0.5 cm-path-length quartz cuvette containing 1.5 ml of HBS prewarmed to 37 °C. The cuvette was placed in a Perkin–Elmer 3000 fluorescence spectrophotometer, and fluorescence changes were measured and calibrated to $[Ca^{2+}]_i$ as previously described (Jackson *et al.*, 1988), by using excitation and emission wavelengths of 340 nm and 500 nm respectively (Grynkiewicz *et al.*, 1985). Peptides were from Cambridge Research Biochemicals (bradykinin, [Thi^{5,8}, D-Phe^7]bradykinin) or from Peninsula Laboratories [Lys-bradykinin (kallidin), des-Arg^9-bradykinin].

Assay for [^{3}H]inositol monophosphate accumulation

Chondrocytes were plated in 35 mm dishes in 2 ml of inositol-free DMEM containing 5% dialysed FCS and 10 μCi of [^{3}H]inositol (NEN)/ml. After a labelling period of 72 h, medium was removed, cells were washed with HBS and incubated in fresh prewarmed HBS containing appropriate concentrations of bradykinin and 10 mM-LiCl (Sigma), to block the conversion of inositol monophosphate into inositol (Berridge *et al.*, 1982). After a period of 30 min the incubation was terminated by addition of an equal volume of 10% (w/v) $HClO_4$, and [^{3}H]inositol phosphate and [^{3}H]inositol-lipid-containing fractions were extracted as previously described (Jackson *et al.*, 1988). [^{3}H]Inositol monophosphates were then separated from labelled free inositol, glycerophosphoinositol and remaining inositol polyphosphates by sequential elution from 0.5 ml columns of Dowex AG1X8, in 2 × 5 ml fractions of distilled water (eluting free [^{3}H]inositol), 2 × 5 ml fractions of 60 mM-ammonium formate/5 mM-disodium tetraborate (eluting [^{3}H]glycerophosphoinositol), and 2 × 5 ml fractions of 0.15 M-ammonium formate/0.1 M-formic acid (eluting [^{3}H]inositol monophosphate). In some experiments, highly labelled chondrocytes were extracted and analysed by h.p.l.c. as described previously (Jackson *et al.*, 1987), to confirm that the earliest change in [^{3}H]inositol phosphate production was an increase in authentic [^{3}H]inositol 1,4,5-trisphosphate. Activity was determined by liquid-scintillation counting.

DNA synthesis, protein synthesis and SDS/polyacrylamide-gel electrophoresis

The rate of DNA synthesis was determined as uptake of [^{3}H]thymidine into trichloroacetic acid-precipitable material. Chondrocytes in 24-well plates were labelled for 24 h with 2 μCi of [^{3}H]thymidine (NEN)/ml in the absence and the presence of appropriate concentrations of drug. Medium was removed and cold trichloroacetic acid added to each well and incubated for 20 min on ice. Cell layers were washed twice with ethanol and taken up into 0.5 ml of 0.1 M-NaOH/2% (w/v) $NaHCO_3$. The radioactivity in each fraction was determined by liquid-scintillation counting.

The rate of total protein synthesis was determined as uptake of [^{35}S]methionine into trichloroacetic acid-precipitable material. Chondrocytes were incubated with 10 μCi of [^{35}S]methionine/ml for 24 h. At the end of the incubation, medium was removed and the cell layer washed with PBS and solubilized in 0.25% SDS in 0.1 M-Tris/HCl buffer, pH 6.8. Fractions of medium and solubilized cell layer were spotted on to squares of Whatman no. 1 paper, and radiolabelled proteins were precipitated by soaking twice in cold 5% trichloroacetic acid for 10 min, followed by two rinses in cold ethanol and a final rinse in acetone. Paper squares were dried and radioactivity was determined by liquid-scintillation counting. Appropriate fractions of the labelled cell-layer preparations were used for SDS/polyacrylamide-gel electrophoresis. Samples were run on a 7.5%-acrylamide discontinuous gel system (Laemmli, 1970). Gels were processed for fluorography, dried and exposed to pre-flashed X-ray film at −80 °C (Bonner & Laskey, 1974).

Proteoglycan synthesis and resorption

The rate of synthesis of proteoglycan in chondrocyte cultures was assessed as uptake of [^{35}S]sulphate into glycosaminoglycan as described by Tyler (1985). The ability of bradykinin to stimulate cartilage resorption was assessed by determining the release of unlabelled glycosaminoglycan from pig cartilage explant cultures by using the dye dimethyl-Methylene Blue (Farndale *et al.*, 1982). For explant cultures, strips from the condylar ridge of pig articular cartilage were cut into pieces approx. 10 mm long. Cultures containing approx. 200 mg of cartilage were incubated in 5 ml of Iscove's modified DMEM containing 400 μg of bovine serum albumin/ml, 5 μg of human transferrin/ml, 100 μg of ascorbic acid/ml and 50 μg of gentamycin/ml. Explants were incubated for 3 days before addition of bradykinin and for a further 3 days in the presence of increasing doses of bradykinin before assessment of glycosaminoglycan release.

PGE_2 assay

PGE_2 was measured in culture media by radioimmunoassay by a method adapted from Levine *et al.* (1971). PGE_2 antiserum and standard were from Steranti Research. Bound and free fractions were separated by using dextran-coated charcoal. Chondrocytes were cultured in 35 mm dishes for 48 h, as described above. Medium was then removed and replaced with 0.5 ml of serum-free DMEM containing increasing doses of bradykinin and cultured for a further 24 h.

RESULTS

Bradykinin elevates $[Ca^{2+}]_i$ in primary chondrocytes

Addition of bradykinin (1 μM) to primary pig chondrocytes, loaded with Fura-2, produced a rapid rise in $[Ca^{2+}]_i$ from basal values of 180 ± 30 nM ($n = 8$) to peak values of 330 ± 50 nM ($n = 8$). A similar increase was seen on addition of 1 μM-Lys-bradykinin (kallidin), a naturally occurring *N*-terminally extended form of

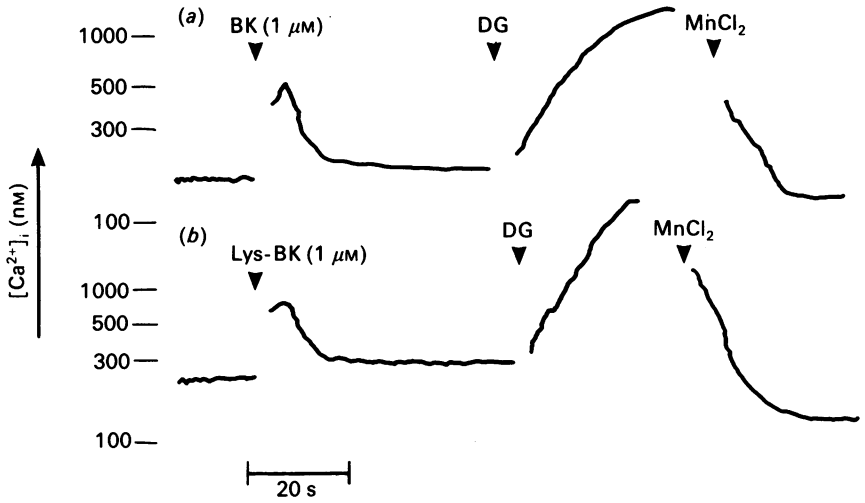

Fig. 1. $[Ca^{2+}]_i$ transients in response to bradykinin (*a*) or lysyl-bradykinin (*b*)

Representative transient $[Ca^{2+}]_i$ responses to bradykinin (BK) or lysyl-bradykinin (Lys-BK) in the presence of 1 mM extracellular Ca^{2+} in Fura-2-loaded chondrocytes (excitation wavelength 340 nm, emission wavelength 500 nm) are shown. Maximal fluorescence and basal autofluorescence were determined by addition of digitonin (DG) or $MnCl_2$ respectively as described in the Materials and methods section.

Table 1. Effects of bradykinin, bradykinin analogues and a bradykinin antagonist on $[Ca^{2+}]_i$ in chondrocytes

The time course of $[Ca^{2+}]_i$ in chondrocytes preloaded with Fura-2 was studied as described in the Materials and methods section. The Table shows responses to bradykinin, bradykinin analogues and the blocking action of a bradykinin antagonist in the presence and absence of extracellular Ca^{2+}. Values are means ± S.E.M. for three to nine determinations.

In the presence of:	$[Ca^{2+}]_i$ basal (nM)	$[Ca^{2+}]_i$ peak (nM)	Time to peak (s)
1 mM extracellular Ca^{2+}			
1 μM-BK	178±28	332±49	5.2±0.8
1 μM-Lys-BK	190±66	390±70	5.4±0.4
1 μM-Des-Arg^9-BK	160±35	153±68	–
1 μM-BK after addition of 10 μM-[Thi^{5,8},D-Phe^7]-BK	172±49	183±35	–
1 mM extracellular EGTA			
1 μM-BK	154±59	371±42	6.1±0.7
1 μM-Lys-BK	163±64	351±36	5.4±0.6

bradykinin (see Fig. 1). The rise in $[Ca^{2+}]_i$ reached a peak within 5–6 s. Table 1 shows that the pharmacological analogue of bradykinin, des-Arg^9-bradykinin, failed to produce an elevation of $[Ca^{2+}]_i$. Addition of [Thi^{5,8},D-Phe^7]bradykinin (10 μM), a bradykinin antagonist (Vavrek & Stewart, 1985), 10 s before the addition of bradykinin (1 μM) blocked the rise in $[Ca^{2+}]_i$. In the absence of extracellular Ca^{2+} and in the presence of 1 mM-EGTA, bradykinin and Lys-bradykinin caused rises in $[Ca^{2+}]_i$ which were not significantly different from those seen in the presence of extracellular Ca^{2+} (Table 1).

No response was obtained if the same coverslip of chondrocytes was challenged a second time with bradykinin, which may arise as a result of depletion of the releasable store of intracellular Ca^{2+} or desensitization of the bradykinin receptor (Jackson *et al.*, 1987).

A large number of other mediators did not cause a rise in $[Ca^{2+}]_i$ in Fura-2-loaded chondrocytes. These included two mediators reported to act in chondrocytes: interleukin 1, which has extensive functional effects on chondrocyte biology, but acts via a currently unknown mechanism, and histamine, suggesting that previously observed effects of this agent (Taylor *et al.*, 1985, 1986) must proceed through adenylate cyclase-linked H2 receptors and not through Ca^{2+}-mobilizing H1 receptors.

Bradykinin increases inositol monophosphate accumulation in LiCl-treated chondrocytes

Fig. 2 shows that bradykinin caused a dose-dependent [$ED_{50} \sim 40$ nM] accumulation of [^{3}H]inositol monophosphate after incubation in the presence of LiCl for 30 min. No significant changes were seen in the [^{3}H]inositol-labelled lipid fraction or in the glycerophosphoinositide or higher inositol polyphosphate fractions (results not shown). In a representative experiment, bradykinin (1 μM) gave increased inositol monophosphate accumulation, from a control value of 2398±410 c.p.m. ($n = 3$) to a stimulated value of 4137±472 c.p.m. ($n = 3$).

When incubations were carried out in the presence of indomethacin, there was no significant change in the bradykinin-stimulated accumulation of inositol monophosphate.

Bradykinin increases PGE_2 secretion by chondrocytes

High-density chondrocyte cultures were incubated in DMEM containing 5% FCS for 48 h and then for a further 24 h in serum-free medium containing increasing doses of bradykinin. Radioimmunoassay of medium showed a stimulation in the concentration of PGE_2 (Table 2). This was significant ($P < 0.05$ compared with control) at concentrations of 100 nM and greater. Addition of indomethacin (5 μM) decreased PGE_2 secretion to undetectable amounts (< 0.05 ng/ml) in both control and bradykinin-treated cultures.

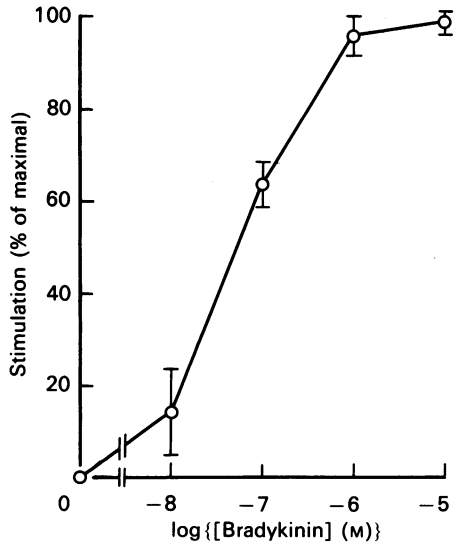

Fig. 2. Inositol monophosphate accumulation in LiCl-treated chondrocytes in the presence of increasing concentrations of bradykinin

Chondrocytes were prelabelled with [^{3}H]inositol as described in the Materials and methods section, and stimulated with increasing doses of bradykinin for 30 min in the presence of 10 mM-LiCl. Values represent means ± S.E.M. for three to four separate determinations, each performed in triplicate. Maximal stimulation was in the range 3000–4500 c.p.m. and basal values were in the range 1500–2500 c.p.m.

Table 2. Effects of inceasing doses of bradykinin on PGE_2 secretion by chondrocytes

Chondrocytes were cultured as described in the Materials and methods section in the presence of increasing concentrations of bradykinin (BK). PGE_2 in the medium was determined by radioimmunoassay. Values represent means ± S.E.M. for four separate determinations, each performed in duplicate.

Treatment	PGE_2 (ng/ml)
Control (DMEM alone)	0.32 ± 0.18
10 nM-BK	0.56 ± 0.27
100 nM-BK	0.94 ± 0.33
1 μM-BK	2.63 ± 0.51
10 μM-BK	2.94 ± 0.61

Bradykinin does not increase the rate of chondrocyte DNA synthesis and glycosaminoglycan synthesis or stimulate cartilage resorption

Bradykinin (0.01–10 μM) did not stimulate entry into DNA synthesis or the rate of glycosaminoglycan synthesis in chondrocyte cultures, as measured by the rates of uptake of [^{3}H]thymidine and [^{35}S]sulphate respectively.

The rate of loss of total glycosaminoglycan from the matrix of cartilage explant cultures into the medium was also unaltered by bradykinin. The total glycosaminoglycan content of cartilage explants was 658 ± 54 μg (78 ± 22 μg in the medium) in control cultures and 634 ± 69 μg (67 ± 15 μg in the medium) in explants treated with bradykinin (1 μM).

Table 3. Effects of bradykinin and thapsigargin on $[Ca^{2+}]_i$ in chondrocytes

The time courses of $[Ca^{2+}]_i$ in chondrocytes were studied in the presence of bradykinin (BK, 1 μM), or thapsigargin (TG, 1.7 μM) after prior addition of bradykinin (TG after BK) or thapsigargin (TG, 1.7 μM) in the presence and absence of extracellular Ca^{2+}. Values represent means ± S.E.M. for three to eight separate determinations.

In the presence of:	$[Ca^{2+}]_i$ basal (nM)	$[Ca^{2+}]_i$ peak (nM)	Time to peak (s)
1 mM extracellular calcium			
BK	163 ± 19	359 ± 24	5.32 ± 0.68
TG after BK	207 ± 21	342 ± 56	15.60 ± 4.10
TG	176 ± 37	318 ± 47	16.00 ± 3.20
1 mM extracellular EGTA			
TG	145 ± 27	356 ± 79	17.20 ± 80

Thapsigargin stimulates $[Ca^{2+}]_i$ in chondrocytes

Addition of thapsigargin (1.7 μM) to chondrocytes loaded with Fura-2 caused a significant elevation in $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+}, from basal values of 180 ± 40 ($n = 4$) to peak values of 320 ± 50 ($n = 4$). A similar stimulation was seen in the absence of extracellular calcium, $[Ca^{2+}]_i$ reaching a peak value of 360 ± 80 nM ($n = 3$) from a basal value of 145 ± 30 nM (Table 3). The magnitude of this response was not significantly different from the magnitude of the response to bradykinin. However, the time courses of the two responses were different (Table 3), the peak of the bradykinin response occurring within 6 s, compared with a significantly slower rise in $[Ca^{2+}]_i$ for thapsigargin, a peak not being reached until 16 s after treatment. Unlike bradykinin, thapsigargin appeared to establish a sustained rise in the steady-state $[Ca^{2+}]_i$.

Table 3 also demonstrates the response if the same chondrocyte population is challenged with both bradykinin and thapsigargin. When chondroctyes are treated with bradykinin, after recovery, a response to thapsigargin may be obtained (Fig. 3*a*). However, thapsigargin abolishes the response to bradykinin, indicating a degree of overlap between the releasable pools sensitive to either agent.

Both Ca^{2+} ionophore A23187 and thapsigargin, but not bradykinin, alter the pattern of protein synthesis by chondrocytes

Chondrocytes were cultured for 48 h and then labelled with [^{35}S]methionine in the presence of bradykinin, thapsigargin or Ca^{2+} ionophore. The cell layer was solubilized and run on SDS/7.5 %-polyacrylamide gels as described in the Materials and methods section. Fig. 4 shows that bradykinin caused no detectable change in the pattern of newly synthesized proteins. However, both ionophore and thapsigargin inhibited the synthesis of a number of cellular proteins, while greatly stimulating the synthesis of a protein of M_r around 80000. This provides

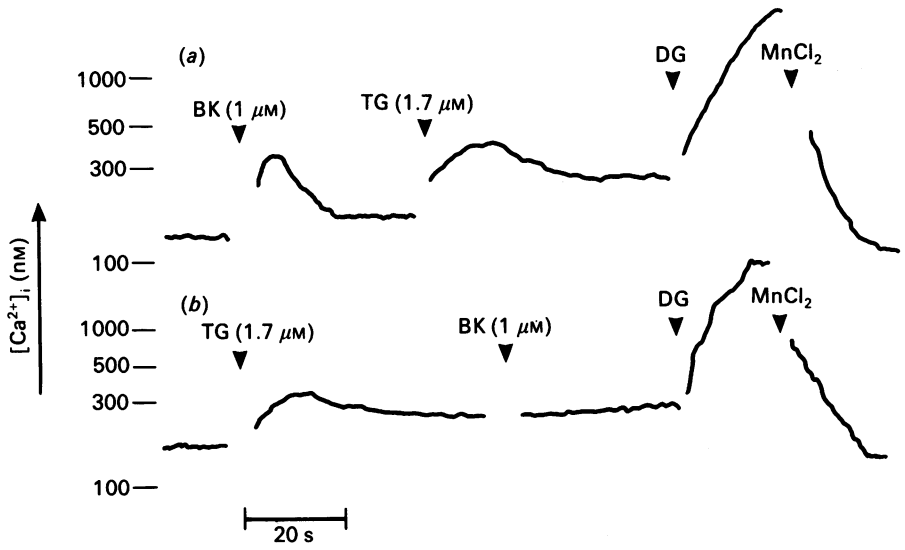

Fig. 3. $[Ca^{2+}]_i$ transients in response to bradykinin and thapsigargin

(*a*) Representative transient elevations of $[Ca^{2+}]_i$ in response to a maximal concentration of bradykinin (BK) followed by a maximal concentration of thapsigargin (TG). (*b*) Representative transient elevations of $[Ca^{2+}]_i$ in response to thapsigargin (TG) followed by bradykinin (BK). Digitonin (DG) and $MnCl_2$ were added to determine maximal and minimal fluorescence as described in the Materials and methods section.

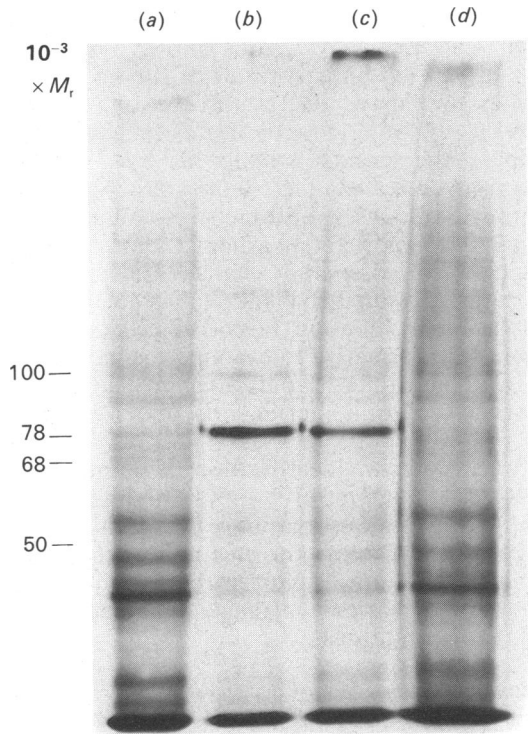

Fig. 4. SDS/7.5%-polyacrylamide gel showing [^{35}S]methionine-labelled proteins synthesized by chondrocytes

Chondrocytes were labelled with [^{35}S]methionine, solubilized and electrophoresed on SDS/polyacrylamide gels as described in the Materials and methods section. Lanes: (*a*) chondrocytes from control cultures, (*b*) chondrocytes cultured in the presence of A23187 (1 μM), (*c*) chondrocytes cultured in the presence of thapsigargin (1.7 μM), (*d*) chondrocytes cultured in the presence of bradykinin (1 μM). Calibration for M_r is shown on the left.

evidence that the hormonal elevation of $[Ca^{2+}]_i$ in chondrocytes caused by bradykinin is not sufficient to mimic the pharmacological responses to addition of ionophore or thapsigargin.

DISCUSSION

Among the identified signal-transduction pathways, the receptor regulation of inositol lipid hydrolysis, and its associated consequences of intracellular Ca^{2+} mobilization and protein kinase C activation, have not to date been demonstrated in chondroctyes. However, the ubiquity of inositol-lipid signalling across vertebrate cell types (Berridge, 1987) has encouraged examination of chondrocyte sensitivity to candidate stimulants of this cascade. Upon screening of a panel of such candidates, functional bradykinin receptors were detected by using the $[Ca^{2+}]$ indicator, Fura-2, by the characteristic initiation of a cytosolic Ca^{2+}-transient. Articular chondrocytes responded by a transient increase in intracellular Ca^{2+}, which was prevented by the specific bradykinin-receptor antagonist [Thi^{5,8},D-Phe^{7}]-bradykinin. The presence of functional receptors for bradykinin was independently confirmed by the dose-dependent stimulation of inositol phosphate or PGE_2 accumulation. The sensitivity of these responses to the *N*-terminal-extended form of bradykinin, kallidin, and insensitivity to the shortened analogue, des-Arg^{9}-bradykinin, suggested that these receptors were pharmacologically similar to, if not identical with, those previously described on neural cells (Hanley *et al.*, 1988).

The bradykinin-receptor-coupled early events are also very similar to those described previously on neural cells (Jackson *et al.*, 1987), but differ quantitatively. Although the basal values of $[Ca^{2+}]_i$ are in both cases close to a calibrated value of 150 nM, the stimulated rise in $[Ca^{2+}]_i$

was much lower in chondrocytes, corresponding to a value approximately one-half the maximal value elicited by saturating bradykinin in the neural cell population (Jackson *et al.*, 1987). A similar difference is observed in the extent of inositol phosphate accumulation. These results could arise from a lower number of active receptors on the chondrocytes. However, the lower maximal $[Ca^{2+}]_i$ elicited by the inositol phosphate-independent stimulant thapsigargin (Jackson *et al.*, 1988) suggests alternative explanations: that either the releasable store is much less in chondrocytes, or rather there is functional heterogeneity of the chondrocyte population so that only a subset responds to a given stimulant. In this regard, it is important to note that there is clear evidence for morphological (Schenk *et al.*, 1986) and phenotypic (Bayliss *et al.*, 1983) heterogeneity of chondrocytes at different depths in the articular cartilage.

The identification of bradykinin sensitivity on chondrocytes attracts special interest, as bradykinin is a recognized mediator in acute and chronic inflammation, activating a variety of immune cells (Marceau *et al.*, 1983). The kinin family is produced during inflammatory or tissue-injury reactions by the action of tissue or plasma kallikreins on the ubiquitous substrates, kininogens (Muller-Esterl *et al.*, 1986). High concentrations of kinins have been reported in rheumatoid synovial fluid (Keele & Eisen, 1970), suggesting a possible role for this class of peptides in regulating chondrocyte responses in rheumatoid arthritis.

Although the early events after bradykinin-receptor activation are readily observed in chondrocytes, the final cellular responses have proved more subtle and specific than for other inflammatory stimulants of these cells. For example, the major action of another class of inflammatory mediators, the cytokines, on joint tissues is cartilage resorption and inhibition of both the collagen (Tyler & Benton, 1988) and proteoglycan (Benton & Tyler, 1988) matrix synthesis. In pig cartilage explants, we found bradykinin to have no effects on glycosaminoglycan synthesis or release, and thus no actions on net matrix resorption or reconstruction. This contrasts with kinin action in promoting bone resorption (Lerner *et al.*, 1987). Similarly, bradykinin, which has been shown to be a mitogen for other connective-tissue cells (Owen & Villereal, 1983), does not alter the rate of chondrocyte DNA synthesis.

In chondrocytes, the only biological output response that we have detected for bradykinin is a powerful stimulation of the production of the eicosanoid PGE_2. As increased PGE_2 production is detected in a number of inflammatory conditions, including rheumatoid arthritis (Henderson *et al.*, 1987), it is a distinct possibility that kinin stimulation of chondrocytes may contribute to the appearance of this prostanoid in cartilage. The enhanced production of PGE_2 is a final common response in the activation of chondrocytes by at least two other unrelated mediators, interleukin-1 (Martin & Resch, 1988) and histamine (Taylor *et al.*, 1985) suggesting that this may be a specific means of identifying the inflammatory activation of chondrocytes during cartilage damage or infection. A threshold amount of a widely distributed kinin or cytokine can thereby generate amplified focal sources of a chemotactic regulator for the emigration and homing of mobile inflammatory cells (Ziff & Cavender, 1987). One conclusion may be that kinin production could be an early event in joint inflammation.

We approached the possible regulatory functions of increased Ca^{2+} in a manner independent of hormonal activation, by using the established Ca^{2+} ionophore, A23187, and the potentially more physiologically relevant probe, thapsigargin, which induces a direct discharge of stored intracellular Ca^{2+} (Jackson *et al.*, 1988; Hanley *et al.*, 1988). Both A23187 and thapsigargin dramatically alter the pattern of new protein synthesis, causing a specific induction of an M_r-80000 band against a background of decreased protein synthesis. This pattern of effects is similar to that induced in a variety of cells by environmental stress, such as heat shock or glucose starvation (Lee, 1987). However, these events are not elicited by increasing Ca^{2+} to a similar peak value by a physiological stimulant, bradykinin. One possibility is that bradykinin may also activate protein kinase C, and that the consequences of this activation may alter the cellular response to $[Ca^{2+}]_i$ elevation. However, the simultaneous addition of phorbol diester with either thapsigargin or A23187 does not alter the induction of the M_r-80000 band (results not shown). Thus it is unlikely that the different consequences of hormonal versus pharmacological stimulation of cytosolic Ca^{2+} increases can be explained by a modulatory influence of protein kinase C.

Two conclusions follow from this observation. First, although A23187 and thapsigargin may be valuable molecular probes of Ca^{2+} homoeostasis mechanisms in chondrocytes, they cannot be used to deduce the physiological consequences of hormonal activation through Ca^{2+} pathways. Thus earlier reports of the actions of A23187 on chondrocytes (Eilam *et al.*, 1985; Malemud & Papay, 1982) must be interpreted with caution.

Second, the events stimulated by A23187 and thapsigargin may in fact more closely mimic the chondrocyte responses to severe stress. When embedded in the cartilage matrix of an articular joint, chondrocytes are subject to dramatic changes in shear forces, hydrostatic pressure and nutrient availability which would be highly unusual for other cell types. It may therefore be informative to examine the mechanisms by which Ca^{2+} and other signals alter protein synthesis in these specialized cells, particularly under conditions of stress.

In conclusion, the identification of both a hormonal mediator of the inositol lipid pathways, and a physiologically relevant output response to activation of these events, may be useful starting points for a closer examination of the involvement of inositol lipid metabolism in normal cartilage maintenance and in pathological conditions.

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