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Insulin-like growth factors counteract the effect of interleukin 1β on type II phospholipase A2 expression and arachidonic acid release by rabbit articular chondrocytes

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Abstract

Interleukin 1β was found to stimulate arachidonic acid release, and the synthesis and secretion of type II phospholipase A2 by rabbit articular chondrocytes in vitro. Interleukin 1β had no effect on the level of cytosolic phospholipase A2 mRNA. Insulin-like growth factors, which help stabilize the cartilage matrix, reduced the effect of interleukin 1β on type II phospholipase A2 activity and mRNA level, and decreased the Interleukin 1β-stimulated arachidonic acid release to the basal values. This suggests that type II phospholipase A2 plays a key role in arachidonic acid release from rabbit articular chondrocytes and that insulin-like growth factors counteract the effect of interleukin 1β. They may therefore be considered as potential antiinflammatory agents.

Key words: Phospholipase A2; Arachidonic acid; Chondrocyte; Interleukin 1β; Insulin-like growth factor; Arthritis

1. Introduction

The synthesis of eicosanoids in many cells depends on the availability of free arachidonic acid (AA) and the major source of free AA is its release from membrane phospholipids by a cellular phospholipase A2 (PLA2). This release of AA from membrane phospholipids seems to be the rate-limiting step in production of lipid mediators during inflammatory disorders [1]. Little is known about the identity and the regulation of the PLA2 responsible for the releasing of AA from activated cells. Two main types of cellular PLA2 have been cloned to date. One has a high molecular weight (85 kDa), and is found in the cytosol of such cells as macrophages [2], synoviocytes [3], mesangial cells [4] or fibroblasts [5]. This PLA2 is highly specific for arachidonic acid, and is activated by a receptor-specific pathway [6]. The other type of PLA2 has a low molecular weight (14 kDa) (sPLA2) [7,8] and is secreted by chondrocytes [9,10], synoviocytes [3], and mesangial cells [11].

High amounts of sPLA2 have been found in the synovial fluid [12] and cartilage matrix [13] of patients suffering from joint diseases with local inflammation such as rheumatoid arthritis. It has recently been suggested that the chondrocytes are the main source of this protein [14]. Among the factors which regulate PLA2 production by chondrocytes or other connective tissue cells, interleukin 1β ($IL_1\beta$), a proinflammatory cytokine, has been shown to induce AA release from both chondrocytes [15] and synoviocytes [16], and induce the expression of the sPLA2 gene in chondrocytes [9] and the cPLA2 gene in synoviocytes [3]. $IL_1\beta$ also causes cartilage destruction inducing an imbalance between matrix synthesis and degradation by chondrocytes [17,18].

In contrast, growth factors such as insulin-like growth factor 1 and 2 (IGF_1 and IGF_2), which are also secreted by cartilage and connective tissue cells, favor cartilage differentiation by stimulating chondrocyte matrix production [19]. But no information is available as to whether there is any interaction between IGFs and $IL_1\beta$ in cartilage cells.

This study was therefore designed to investigate whether IGF_1 and IGF_2 interfere with the regulation of inflammatory markers induced by $IL_1\beta$ in chondrocytes. $IL_1\beta$ stimulated the AA release and the expression of sPLA2 gene, but appeared to have no effect on cPLA2. IGF_1 and IGF_2 reduced the effects of $IL_1\beta$ on sPLA2 mRNA level in chondrocytes, the sPLA2 activity, and the amount of AA release.

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Abbreviations: AA: arachidonic acid; IGF: insulin-like growth factor; $IL_1\beta$: interleukin 1β; PLA2: phospholipase A2; sPLA2: secreted PLA2; cPLA2: cytosolic PLA2

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2. Materials and methods

2.1. Chondrocyte culture

Four-week-old rabbits were killed and the shoulders, knees and femoral heads were dissected out under sterile conditions [20]. The articular cartilage was removed and cut into small pieces (< 0.5 mm^2) and digested successively at 37°C with 0.05% testicular hyaluronidase in HAM's F12 medium (from Flobio) for 15 min, 0.25% trypsin for 30 min and 0.2% clostridial collagenase for 90 min. The hyaluronidase, collagenase and trypsin were purchased from Boehringer. The resulting suspension of chondrocytes was seeded into 75 cm^2 flasks (4×10^5 cells per flask) or into 24-well cell culture plates (2×10^4 cells per well) in HAM's F12 supplemented with 10% fetal calf serum, 10 UI/ml penicillin and 100 μg/ml streptomycin. The cells were maintained at 37°C in 5% CO_2, and the culture medium was changed every 2–3 days. The cells reached confluency within 6–7 days. Cells cultured in 24-well plates were used to measure arachidonic acid release, whereas cells in 75 cm^2 flasks were used for mRNA extraction and PLA2 assay.

2.2. Arachidonic acid release

Confluent cells were incubated in a serum-free DMEM medium for 24 h and then with 0.5 μCi/ml [^{3}H]arachidonic acid (3,000 Ci/mmol, Amersham) in DMEM supplemented with 0.1% IGF-free bovine serum albumin (Sigma) at 37°C for 1 h. The cells were washed three times with phosphate-buffered saline. They were then incubated with human recombinant $IL_1\beta$ (0.1–100 ng/ml) (Immugenex, USA), and-or IGF_1 or IGF_2 (10–100 ng/ml) (Genzyme by Tebu, France). In some experiments, cells were incubated with the peptides for periods of 5 min–24 h. Aliquots of the supernatant were removed and the arachidonic acid release measured. At the end of the incubation, the cells were washed, scraped free and sonicated. The radioactivity of cells and media was quantified by scintillation counting. Results are expressed as percentages of the release under basal conditions in order to compare all experiments.

2.3. Phospholipase A2 assay

The phospholipase A2 activity secreted into media was measured on 100 μl samples using 2 nmol of fluorescent substrate (1-palmitoyl 2(10-pyrenyldecanoyl)-Sn glycero-3-monomethyl phosphatidic acid, Interchim) as described by Radvanyi [21] and modified by Pernas [22]. The specificity of the phospholipase A2 activity measured was assessed by the hydrolysis of labeled substrate L3 phosphatidyl ethanolamine, 1-acyl-2-[1-^{14}C]arachidonyl (50 mCi/mmol, Amersham) [23].

2.4. Northern blotting

Total cell RNA was extracted [24] from confluent chondrocytes cultured in 75 cm^2 flasks . The RNA was fractionated on a 1% agarose gel containing 2.2 M formaldehyde and transferred onto a nylon membrane (Hybond N, Amersham, France). RNA was immobilized by baking at 80°C for 2 h. Hybridization was performed with the rapid hyb-buffer kit (Amersham, France) using full-length cDNA probes for human placental 14 kDa type II PLA2 [22] and human cytosolic 85 kDa PLA2 prepared by polymerase chain reaction from U937 cell mRNA according to Clark [2]. A 28S oligonucleotide probe was used as control in the Northern blots [25]. Secreted PLA2 and cPLA2 cDNA probes were labeled with [α-^{32}P]dCTP (Amersham, France, 3,000 Ci/mmol) by random priming labeling system (Amersham, France). The membranes were prehybridized for 15 min, and then hybridized at 65°C for 90 min, washed with 0.1% SDS in 2 × SSC at room temperature, followed by two washes with 0.1% SDS in 0.1 × SSC at 65°C for 15 min each. The filters were then autoradiographed at −80°C for 18–72 h using MP hyperfilm (Amersham, France). The resulting autoradiographs were scanned with a laser densitometer (2202 ultrascan, LKB). Results are expressed as the ratio of 500 bp (sPLA2) optic density (OD) to the corresponding 28S OD. RNA from C_{127} cells overexpressing type II PLA2 and from U937 cells were used as positive control for 14 kDa sPLA2 and 85 kDa cPLA2, respectively.

2.5. Statistical analysis

When necessary, Student's *t*-test was used for statistical analysis.

3. Results

The release of AA was first studied as a marker of the inflammatory effect of $IL_1\beta$ on chondrocytes. Incubation of chondrocytes with $IL_1\beta$ (0.1–100 ng/ml) for 24 h resulted in a dose-dependent increase in AA release (Fig. 1A) which was significant at 25 and 50 ng/ml $IL_1\beta$ (around 200% of the basal release), but not significant between 50 and 100 ng/ml. The decrease at 100 ng/ml could be due to this concentration of $IL_1\beta$ being toxic as shown by a decrease in Trypan blue exclusion (not shown).

Incubation with IGF_1 or IGF_2 alone reduced the release of AA by 25 ± 15% at all the peptide concentrations used (10–100 ng/ml). IGF_1 or IGF_2 (25 or 50 ng/ml) significantly reduced the amount of AA released in response to 25 ng/ml $IL_1\beta$, back to the basal level ($P < 0.05$) (Fig. 1B). $IL_1\beta$ did not stimulate the release of AA before 24 h (not shown). In contrast, the time

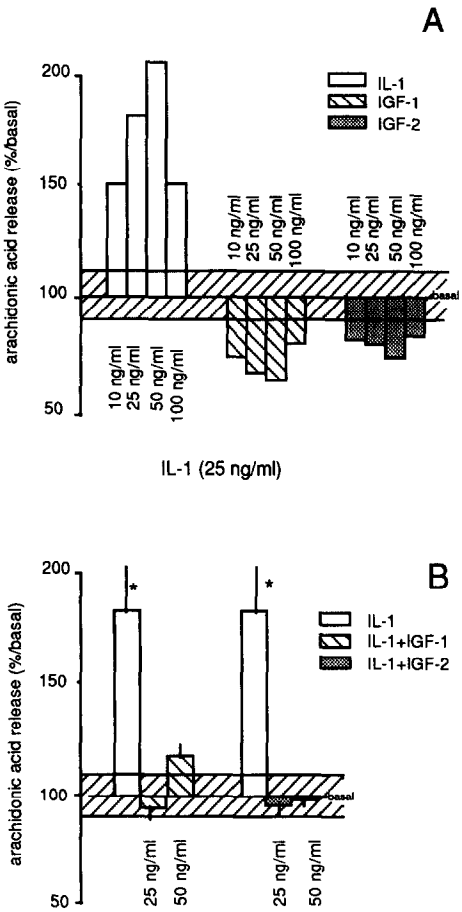


Fig. 1. Effects of $IL_1\beta$, IGF_1 and IGF_2 on arachidonic acid release from prelabelled rabbit chondrocytes. The cells were labelled with [^{3}H]arachidonic acid as described in Section 2. The arachidonic acid released into the medium after a 24 h stimulation by effectors is expressed as% of the release under basal conditions. The shaded areas indicate the basal release (mean ± S.D. of 5 experiments); 100% control cell release corresponds to 13% ± 2% of total radioactivity. (A) The effects of $IL_1\beta$, IGF_1 or IGF_2. (B) The effects of IGF_1 and IGF_2 on arachidonic acid release induced by 25 ng/ml $IL_1\beta$. Data are the means ± S.E.M. of 5 experiments each performed in triplicate. *$P < 0.05$

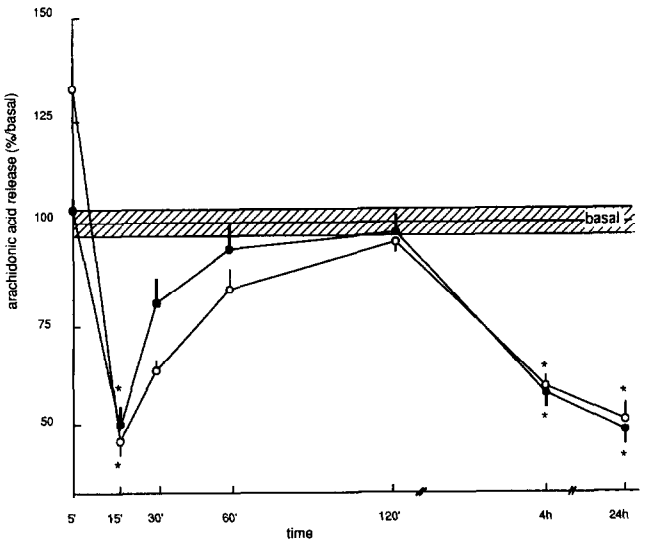


Fig. 2. Time course of the effect of 25 ng/ml IGF_1 (○) and 25 ng/ml IGF_2 (●) on the basal arachidonic acid release. Values are the means ± S.D. of 2 experiments each performed in triplicate. The shaded areas indicate the basal release (means ± S.D. of 5 experiments). The corresponding control releases were: 3% ± 1.7% for 30 min; 4.1% ± 1.9% for 60 min; 7.9% ± 4.4% for 120 min; 10.2% ± 1.9% for 4 h and 13% ± 2% for 24 h.

course of the effects of IGF_1 or IGF_2 on AA release appeared to be biphasic: a sharp and rapid decrease down to 50% below the control values occurred 15 min after adding the peptides and AA release returned to the basal level between 60 and 120 min. A second decrease occured 4 h later, reaching a minimum (50% of control) at 24h (Fig. 2).

Chondrocyte mRNA PLA2 transcripts hybridized with two specific probes, one identifying sPLA2 mRNA expressed as a 500 bp band in C127 transfected cells and the other identifying cPLA2 mRNA expressed as a 3200 bp band in U937 cells (Fig. 3). No chondrocyte sPLA2 mRNA was detected under basal conditions, whereas cPLA2 mRNA was clearly observed. Incubation with 10 ng/ml $IL_1\beta$ for 24 h stimulated production of sPLA2 mRNA, but did not alter the cPLA2 mRNA level. The chondrocyte cPLA2 mRNA was not studied further. The expression of sPLA2 mRNA induced by $IL_1\beta$ was dose-dependent between 0.1 to 10 ng/ml (Fig. 4) after incubation for 4 h and 24 h with $IL_1\beta$. The sPLA2 signal was decreased by incubation with 25 ng/ml IGF_1 or IGF_2 plus 10 ng/ml $IL_1\beta$ (Fig. 4B). This inhibitory effect of IGF_1 or IGF_2 was more pronounced after 4 h (35% and 54%, respectively) than after 24 h (20% and 10%, respectively). Secreted PLA2 enzymic activity was not detected in the culture media of chondrocytes incubated under basal conditions (Fig. 5). Cells incubated with 10 to 50 ng/ml $IL_1\beta$ for 24 h released considerable sPLA2 activity into the culture medium. IGF_1 or IGF_2 (25 ng/ml) significantly reduced (−65%) the sPLA2 activity induced by 25 ng/ml $IL_1\beta$ alone ($P < 0.001$).

4. Discussion

$IL_1\beta$ had parallel effects on AA release, sPLA2 mRNA level and sPLA2 activity in rabbit articular chondrocytes. These results are consistent with those reported by others [9,10] for similar cell types. Hulkower et al. [3] recently found that no increase on sPLA2 activity could account for the increased PGE_2 production induced by $IL_1\beta$ in synovial fibroblasts. They showed that $IL_1\beta$ stimulated cPLA2 activity in parallel with PGE2 release. A similar result was found in rat mesangial cells stimulated by $IL_1\beta$ [4]. But this does not seem to be the case in rabbit articular chondrocytes, as $IL_1\beta$ had no effect on the cPLA2 mRNA level after 24 h (Fig. 3), or on arachidonyl-specific PLA2 activity in the cytosol (not shown). This seems to rule out the involvement of cPLA2 in the stimulation of AA release by $IL_1\beta$ in rabbit articular chondrocytes. However, the $IL_1\beta$-stimulated AA release might not be fully explained by the increase in medium sPLA2, because sPLA2 alone does not appear to release AA from prelabelled cells [22]. Therefore, $IL_1\beta$, besides inducing sPLA2 synthesis might target another mechanism stimulating AA release. This mechanism might involve another unknown PLA2, or the activation of another pathway increasing the effect of sPLA2 on chondrocytes. Such an amplification of sPLA2-mediated release of AA has been described in response to protein kinase C stimulation in human T lymphocytes [26] and in mouse fibroblasts overexpressing human sPLA2 [22].

The data also demonstrate that IGFs inhibit the release of AA. The basal AA release was significantly reduced within 15 min of adding IGF_1 or IGF_2. The chondrocytes contained detectable amounts of cPLA2 mRNA but not of sPLA2 mRNA under the experimental conditions used. The mechanism of this short term effect of IGFs is still unknown. One of the rapid receptor-mediated mechanism modulating cPLA2 activity seems

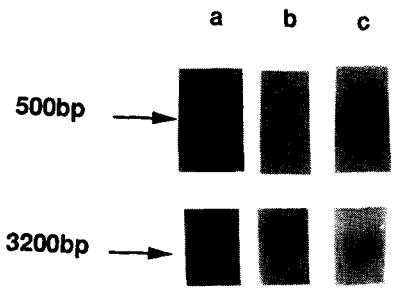


Fig. 3. Northern blot analysis of sPLA2 (500 bp) and cPLA2 (3,200 bp) mRNA in unstimulated and $IL_1\beta$-treated chondrocytes. Lane a: positive controls for sPLA2, mRNA from C_{127} cells transfected by human type II PLA2 and for cPLA2, mRNA from U_{937} cells. Lane b: unstimulated chondrocytes. Lane c: chondrocytes incubated with 10 ng/ml $IL_1\beta$ for 24 h before RNA extraction. 15 μg of total RNA were analysed as described in Section 2. Each lane represents the hybridization of the same blot with the cDNA probes of human sPLA2 and cPLA2, respectively.

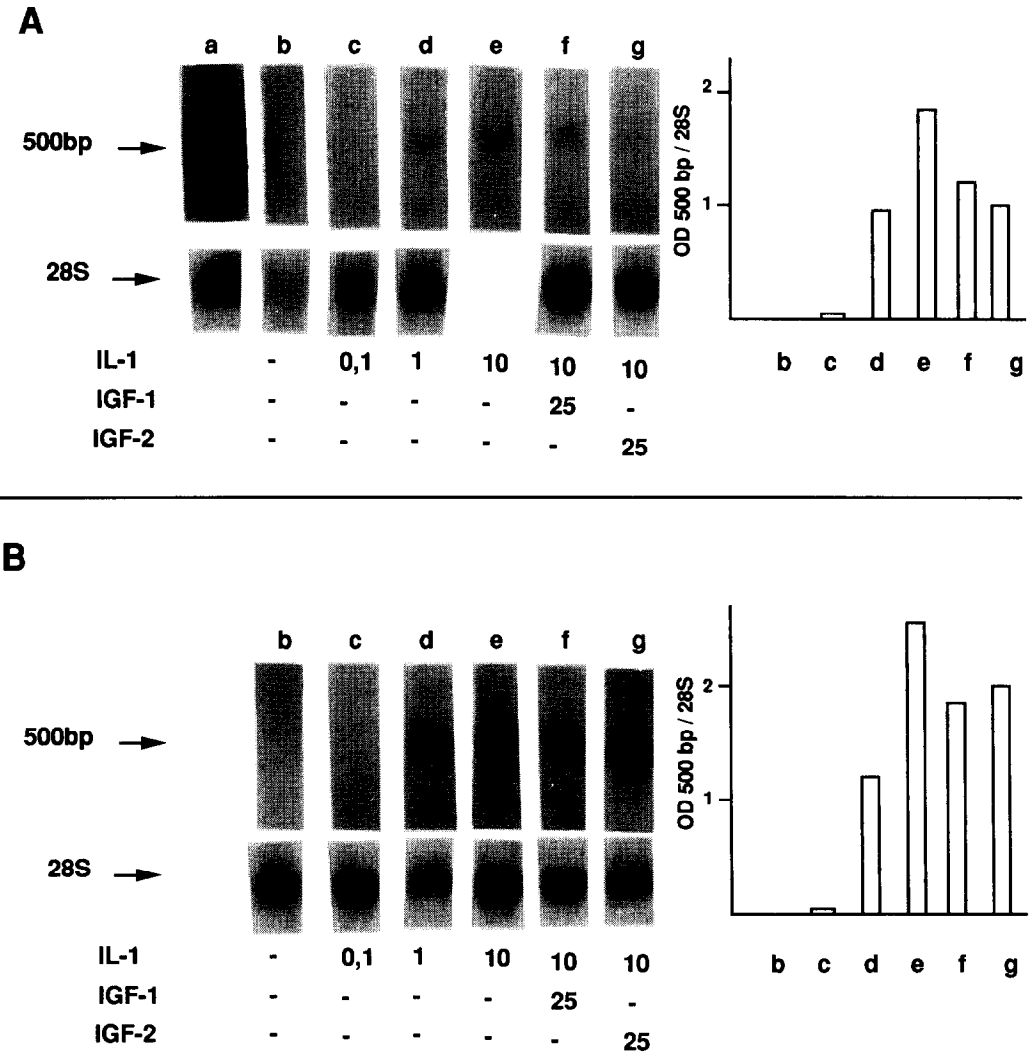


Fig. 4. Effects of $IL_1\beta$, IGF_1 and IGF_2 on mRNA levels of sPLA2. Total RNA was extracted and analysed as described in Section 2. The labelled bands were quantified by scanning the autoradiograms. Data are expressed as the ratio of 500 bp (sPLA2) OD to the corresponding 28 S OD. (A) Effect of a 4h incubation with $IL_1\beta$ and its modulation by IGF_1 and IGF_2. (B) Effect of a 24 h incubation with $IL_1\beta$, and its modulation by IGF_1 and IGF_2. Lane a: positive control for sPLA2: C_{127} cells transfected by human sPLA2. lane b: unstimulated chondrocytes. Lane c: chondrocytes incubated with 0.1 ng/ml IL_1. Lane d: 1 ng/ml $IL_1\beta$. Lane e: 10 ng/ml $IL_1\beta$. Lane f: 10 ng/ml $IL_1\beta$ + 25 ng/ml $IGF_1\beta$. Lane g: 10 ng/ml $IL_1\beta$ + 25 ng/ml IGF_2.

to be its phosphorylation by mitogen-activated protein kinase [6]. IGF_1 might cause negative modulation of chondrocyte cPLA2 by a phosphorylation-dephosphorylation mechanism.

Lastly, incubation of chondrocytes with IGF_1 or IGF_2 for 24 h inhibited the $IL_1\beta$-induced sPLA2 gene expression and enzyme activity, as well as AA release. Further experiments are needed to elucidate the mechanism by which $IL_1\beta$ is inhibited. It could involve inhibition of $IL_1\beta$ receptor synthesis, as occurs with Transforming Growth Factor β (TGFβ) [27], or an interaction between the signalling pathways of IGF1 and $IL_1\beta$-modulating sPLA2 synthesis and secretion. This is, to our knowledge, the first demonstration that both IGF peptides interfere with induced inflammatory processes at concentrations which are known to stimulate chondrocyte matrix production [19,28].

Some of the negative effect of $IL_1\beta$ on joint inflammation could be due to the increased synthesis of sPLA2 by articular chondrocytes, as sPLA2 can induce experimental arthritis [29,30]. It is therefore possible that IGFs, in addition to their anabolic effect on cartilage, act as anti-inflammatory or anti-arthritis factors in joints.

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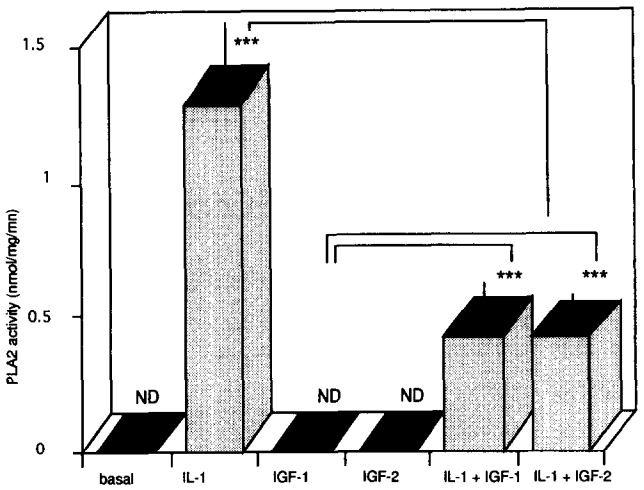


Fig. 5. PLA2 released into the medium from cells incubated with $IL_1\beta$, IGF_1 and IGF_2. The chondrocytes were incubated with the agents for 24 h, the medium was removed, and the PLA2 activity was measured as described in Section 2. Data are the means ± S.E.M. of 3 experiments performed in triplicate. ND = not detectable. ***$P < 0.001$.

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