Serotonin-stimulated phospholipase A2 and collagenase activation in chondrocytes from human osteoarthritic articular cartilage

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We have previously described several receptors on the chondrocyte membrane. In an attempt to further characterize the coupling mechanisms of serotoninergic receptors, here we examined the involvement of serotonin in the phospholipase A2 activity. Serotonin dose-dependently stimulated phospholipase A2. This activation enhanced collagenase type II activity and had no effect on proteoglycanase activity.

Chondrocyte; Serotonin; Phospholipase A2; Collagenase; Osteoarthritis

1. INTRODUCTION

Chronic progressive destruction of the articular joint structure is a characteristic pathological feature of osteoarthritis. Intra-articular elevations of inflammatory mediators and tissue hydrolyzing proteases (collagenase and proteoglycanase) are believed to be responsible for this articular degeneration process [1,2]. The regulation of chondrocyte metabolism is poorly understood but the role of some agents such as histamine [3–6] and interleukin-1 [7] in cartilage degradation is now established. Stimulation of degradative enzymes by chondrocytes has been suggested to be related to prostaglandin synthesis via activation of phospholipase A2 (PLA2) by both interleukin-1 [8] and mechanical trauma [9]. We recently reported that serotoninergic receptors were significantly increased in osteoarthritic cartilage [10]. In the present work serotonin influence on PLA2 activity was studied and correlated with proteoglycanase and collagenase activity.

2. MATERIALS AND METHODS

2.1. Chondrocyte isolation and stimulation

Human osteoarthritic cartilage was obtained from femoral heads or knee joints at the time of total hip or knee replacement (average age 64, range 56–72). At the time of surgery, each specimen was rinsed with physiological saline to remove blood and full depth cartilage was excised aseptically and used immediately. Articular chondrocytes were isolated by sequential enzymatic digestion (trypsin and col-

lagenase) of cartilage slices as described [11,12]. The cells were washed twice with DMEM medium to eliminate collagenase (no collagenolytic activity was found in the last supernatant). The chondrocytes were seeded (4×10^5/well) into 24-well microtiter plates and treated in an incubator at 37°C (atmosphere of 95% air, 5% CO_2) for 60 min with different compounds which were added to the DMEM media.

2.2. Preparation of enzymatic fraction

The chondrocytes were resuspended in 20 mmol/l Tris-HCl buffer, pH 7.40 (10^6 cells/ml buffer). Cells were homogenized with a Potter-Elvehjem and broken up by ultrasonic treatment in ice with a probe (Sonimass type 25 T, 120 V, 4×30 s). The homogenate was used for enzymatic determinations. In acellular experiment systems, compounds were added to the chondrocyte homogenate during enzymatic kinetics.

2.3. Assay for PLA2 activity

The assay was performed using the method of Higuchi et al. [13] with some modifications [14]. Unlabelled L-α-phosphatidylethanolamine and labelled 1-palmitoyl-2-[^{14}C]-linoleyl-L-3-phosphatidylethanolamine were employed as substrates.

2.4. Assay for collagenolytic activity

Collagenolytic activity was determined using [^3H]acetylated soluble type II collagen as a substrate [14]. Collagen was [^3H]acetylated with [^3H]acetic anhydride [15]. Using polyacrylamide gel electrophoresis the labelled collagen molecules degradated by chondrocyte homogenate and a pure collagenase were found to be similar, while pronase digestion gave different products.

2.5. Assay for proteoglycanase activity

Proteoglycans extracted from normal human articular cartilage [14] were [^3H]acetylated [15]. Proteoglycanase was measured by the release of soluble radioactive fragments from labelled proteoglycans [14]. No radioactivity was released by a specific chemical desacetylation of glycosaminoglycans from labelled proteoglycans. The measured radioactivity was thus specific to the proteoglycan core protein degradation.

2.6. Protein assay

Total protein content was determined using the bicinchoninic acid protein [16].

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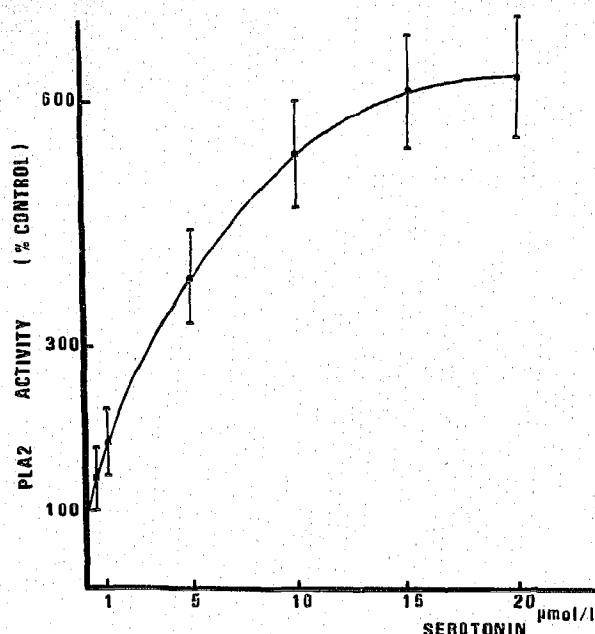
Fig. 1. Dose–response relationship between serotonin concentration and PLA2 activity.

3. RESULTS

Serotonin stimulated PLA2 activity in a concentration-related manner with concentrations as low as 1 μmol/l causing a significant increase above control (Fig. 1). The response appeared to be maximal ($\times$6) by 15 μmol/l.

Treatment of chondrocytes with 15 μmol/l serotonin stimulated PLA2 ($\times$6) and collagenase ($\times$4) activities. Serotonin-stimulated PLA2 and collagenase activities were decreased by mianserin, mepacrine and p-bromophenacyl bromide (BPB) (Table I). No compound had any effect on the proteoglycanase activity.

When mepacrine and BPB were introduced in chon-

Table I

Effects of chondrocyte treatments on PLA2, collagenase and proteoglycanase activities

Addition	PLA2	Collagenase	Proteo-glycanase
None	100	100	100
Serotonin	612 ± 73	405 ± 81	150 ± 64
+Mianserin	158 ± 52	126 ± 54	115 ± 35
+Mepacrine	30 ± 12	35 ± 15	93 ± 42
+p-Bromophenacyl bromide	25 ± 20	30 ± 10	84 ± 32

The concentrations of additions were 15 μmol/l; compounds were added to the chondrocytes for 60 min after which the medium was removed and the cells assayed for enzymatic determinations. The data are the means ± SD from 6 experiments, each assayed in triplicate. Results are expressed as a percentage in relation to controls

Table II

Effects of PLA2 inhibitors in acellular systems on PLA2, collagenase and proteoglycanase activities

Addition	PLA2	Collagenase	Proteo-glycanase
None	100	100	100
Mepacrine	110 ± 15	110 ± 35	120 ± 45
p-Bromophenacyl bromide	15 ± 15	94 ± 20	105 ± 30

The concentrations of additions were 15 μmol/l; compounds were added to the chondrocyte homogenate during enzymatic kinetics. The data are the means ± SD from five experiments, each assayed in triplicate. Results are expressed as a percentage in relation to controls

drocyte homogenate (acellular systems) not in cell medium, only PLA2 was decreased with BPB (Table II). Collagenase and proteoglycanase activities were unmodified.

4. DISCUSSION

Serotonin is known to be a mediator of inflammation. We have previously shown that human osteoarthritic articular chondrocytes possess serotoninergic receptors [10]. The results presented herein show, for the first time to our knowledge that human osteoarthritic articular chondrocytes have a pathway for serotoninergic receptor-mediated activation of PLA2 and collagenase. The following findings suggest this affirmation:

(i) serotonin-stimulated PLA2 and collagenase activities were inhibited by the addition of mianserin, a serotoninergic antagonist [17].

(ii) BPB and mepacrine have been identified as inhibitors of PLA2. BPB covalently modifies essential histidine residues associated with the catalytic site on the enzyme [18]. Mepacrine has been shown to inhibit PLA2 activity in cells but not in acellular systems by altering the calcium availability to the enzyme [19]. These compounds decreased collagenase activity in cell experiments but did not affect this activity in acellular experiments, although BPB remained a PLA2 inhibitor. This last finding suggests that collagenase activation was related to serotonin stimulation via PLA2 activation.

The chondrocyte mechanisms involved in the coupling of serotonin receptor interaction with PLA2 activation are completely unknown. PLA2 is thought to play a central role in providing arachidonic acid for subsequent metabolism to prostaglandin and leucotrienes, potent lipid mediators of inflammation. PLA2 regulation was studied in different models: rabbit platelets with histamine [20], glomerular mesangial cells with epidermal growth factor [21] and macrophage cell line with calcium [22]. Histamine and epidermal growth factor enhance PLA2 activity. Chondrocyte PLA2 ac-

tivation has been described directly with cytokines [7] and indirectly with histamine [23]. The latter shows increased prostaglandin E production with H1 receptor stimulation while the former shows that interleukin-1 induced PLA2 activation, prostaglandin E_2 synthesis and latent neutral protease secretion in rabbit articular chondrocytes. Our results are similar and lead to the suggestion that serotoninergic receptor-mediated activation of PLA2 induced prostaglandin E_2 synthesis which activates collagenolytic activity.

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