Evidence for both histamine H₁ and H₂ receptors on human articular chondrocytes

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SUMMARY Using specific histamine H₁ and H₂ receptor antagonists, evidence is presented for the existence of both H₁ and H₂ receptors on human articular chondrocytes in vitro. Stimulation of the H₁ receptor by histamine (range 0.18 to 17.8 μmol/l) significantly increased prostaglandin E (PGE) production, while activation of the histamine H₂ receptor increased intracellular cyclic adenosine-5'-monophosphate (AMP). The histamine H₁ antagonists mepyramine and triptenolamine blocked the histamine induced increase in PGE production, and the H₂ antagonists cimetidine and ranitidine prevented the increase in intracellular cyclic AMP. These observations suggest that mast cell-chondrocyte interactions mediated via histamine may contribute to some of the pathophysiological changes observed in joint disease.

Key words: adenosine cyclic monophosphate, prostaglandins E.

After our observation of mast cells at sites of cartilage erosion in rheumatoid knee joints we have examined the potential of mast cell components to affect chondrocyte metabolism. The addition of whole mast cell products, prepared from purified rat or dog mast cells, caused a marked increase in the intracellular cyclic AMP levels of cultured chondrocytes, an observation subsequently explained by the demonstration of histamine H₂ receptors on chondrocytes derived from human, canine, and fetal bovine articular cartilage. As with histamine H₂ receptors found in gastric mucosa, rat uterus, and guinea pig heart, stimulation by histamine activated adenylate cyclase and produced an increase in intracellular cyclic AMP. Alterations in intracellular cyclic AMP levels have been shown to affect a number of cellular processes, including prostaglandin (PG) production by human adipose tissue. Since increased PG production is a common feature of inflammatory joint disease we have investigated the effect of histamine on PG production by chondrocytes. We report here that histamine was found to increase PG production by human articular chondrocytes, but surprisingly this process was shown to be mediated not by a histamine H₂ receptor but by a type 1 receptor.

Materials and methods

Materials were obtained from the sources previously given, with the addition of the following: [³H]PGE₂ was obtained from Amersham International, Amersham, Bucks, UK; anti-PGE serum was obtained from Miles Laboratories Limited, Slough, UK; recombinant murine interleukin 1 (IL1) was a gift from Roche Products Ltd, Welwyn Garden City, Herts and was prepared as previously described. (One unit of IL1 activity induces 50% maximal proliferative response in the thymocyte costimulation assay.)

Cell culture

Human articular chondrocytes (HAC) were obtained by proteolytic digestion of macroscopically normal articular cartilage from femoral heads and condyles obtained from remedial surgery as previously described. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% (v/v) fetal calf serum supplemented with penicillin, streptomycin and fungizone. Cultures were incubated at 37°C in CO₂/air 1:19 in a water saturated atmosphere and when confluent subcultured using conventional
trypsin treatment and replating at reduced density. The chondrocyte cultures used in these experiments were from primary and passaged cells, both of which were qualitatively similar in their response to histamine.

CYCLIC AMP STUDIES

For measurement of intracellular cyclic AMP the chondrocytes were incubated in DMEM containing 800 μmol/l isobutyl-1-methylxanthine (IBMX) with and without histamine and antagonists for five minutes at 37°C. After removal of the incubation medium the cells were precipitated with 6% (w/v) HClO₄ and processed for cyclic AMP determination as described previously.⁸ The cyclic AMP was measured by a competitive protein binding assay using the binder isolated from bovine adrenal glands.⁹

PGE STUDIES

To examine the effect of histamine on PGE production chondrocytes were preincubated for between 16 and 24 hours with 10 or 20% (v/v) synovial factor (SF), which has previously been reported to stimulate chondrocytic PG production.¹⁰ This overcame the problem of very low basal PGE production, which was normally at the limit of assay sensitivity. SF was the medium removed after three days from a primary culture of adherent rheumatoid synovial cells, known to be a good source of interleukin 1,¹¹ and after centrifugation to remove cellular debris was used without further treatment. Cells were also preincubated for 20 hours with 16 units/ml murine IL 1, which like SF markedly stimulated chondrocyte prostaglandin E₂ production. After removing the activating medium the cells were washed three times with Hanks's balanced salt solution (HBSS) and DMEM was added with or without histamine and its antagonists. This second incubation was for either one or two hours, after which the medium was removed for PGE measurement. The inclusion of indomethacin (14 μmol/l) reduced PGE production to non-detectable levels, which confirmed that PGE obtained during the histamine incubation represented new synthesis and not leakage of preformed material.

PGE was measured in culture media by radioimmunoassay¹² using an antiserum with similar specificity towards the prostaglandins E₂ and F₂, and using dextran coated charcoal to separate bound from free fractions.¹³ The antiserum was diluted in assay buffer (50 mM phosphate, 0.15 M NaCl, 0.1% (w/v) bovine serum albumin, and 0-05% (w/v) sodium azide at pH 7-4) and 100 μl was added to the same volume of sample and [³H]PGE₂. After 90 min at 4°C 1 mg of dextran coated charcoal was added to each tube, which after vortexing was incubated for a further 10 min at 4°C. After centrifugation at 1500 g for 10 min at 4°C the supernatant was decanted and counted in a liquid scintillation spectrometer connected to a Beckman DP5500 curve-fit processor. The sensitivity of the assay was 5 pg when determined by two standard deviations at zero dose.

[³H]MEPYRAMINE BINDING STUDIES

Radioligand binding studies were performed on confluent monolayers of chondrocytes using the method recently described for rheumatoid synovial cells.¹⁴ After a 90 min incubation at 0-4°C with 10 nM [³H]mepyramine the medium was removed and the cells were rapidly rinsed with buffer before detaching by incubation at 37°C with 0-25% (w/v) trypsin. The cells were transferred quantitatively to vials for liquid scintillation counting. All treatments were performed in duplicate or triplicate.

Results

HISTAMINE EFFECTS ON PGE PRODUCTION

Histamine stimulates PGE production by HAC in Table 1 Histamine stimulation of chondrocyte PGE production

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PGE (ng/well/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM</td>
<td>2.60 (0.08)*</td>
</tr>
<tr>
<td>Histamine (0.18 μmol/l)</td>
<td>2.79 (0.09)*</td>
</tr>
<tr>
<td>Histamine (1.8 μmol/l)</td>
<td>4.64 (0.11)*</td>
</tr>
<tr>
<td>Histamine (17.8 μmol/l)</td>
<td>5.24 (0.09)*</td>
</tr>
<tr>
<td>+ Mepyramine (1.3 μmol/l)</td>
<td>2.17 (0.05)</td>
</tr>
<tr>
<td>+ Cimetidine (40 μmol/l)</td>
<td>3.40 (0.20)*</td>
</tr>
</tbody>
</table>

The chondrocytes were preincubated for 24 h with 20% (v/v) SF. The activating medium was removed and the cells were washed with HBSS before adding histamine and its antagonists for one hour.

*p<0.01.

Values are the mean (SEM). n=4.

Table 2 Histamine stimulation of PGE production from chondrocytes preincubated with SF or murine IL 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PGE (ng/well/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM</td>
<td>6.13 (0.05)*</td>
</tr>
<tr>
<td>Histamine (4.5 μmol/l)</td>
<td>15.81 (0.97)*</td>
</tr>
<tr>
<td>+ Mepyramine (0.5 μmol/l)</td>
<td>5.60 (0.64)</td>
</tr>
<tr>
<td>+ Triptenamine (0.5 μmol/l)</td>
<td>4.75 (0.29)</td>
</tr>
<tr>
<td>+ Cimetidine (7.9 μmol/l)</td>
<td>11.74 (0.51)</td>
</tr>
<tr>
<td>+ Ranitidine (6.3 μmol/l)</td>
<td>14.21 (0.38)*</td>
</tr>
</tbody>
</table>

The chondrocytes were preincubated for 20 h with either 10% (v/v) SF or 16 units/ml murine IL 1. The activating medium was removed and the cells were washed with HBSS before adding histamine and its antagonists for one hour.

*p Values significantly increased above control at p<0.01.

Values are the mean (SEM). n=3.
concentration related manner, with concentrations as low as 0.18 μmol/l causing a significant increase above control (Table 1). In four preparations of HAC, histamine (17.8 μmol/l) increased PGE production by an average of 5.4 (range 2.3–11.3) times that of the control. The stimulation was prevented by the H₁ receptor antagonists mepyramine and tripepallenamine, was not significantly reduced by a 10-fold higher concentration of the H₂ antagonist ranitidine, and only slightly reduced by cimetidine (Tables 1 and 2).

The H₁ antagonists chlorpheniramine, mepyramine, and tripepallenamine each caused a dose related inhibition of the histamine stimulated PGE production at concentrations below 10⁻⁶ mol/l (Fig. 1). When added without histamine the highest concentration of histamine antagonists used in these experiments did not significantly change the control values of PGE production (result not shown). The PGE response of histamine stimulated HAC was approximately doubled after preincubation with either murine recombinant IL1 or synovial factor (SF), an increase prevented by H₁ but not H₂ receptor antagonists (Table 2).

\[ ^3H \text{mepyramine binding to HAC} \]

Five preparations of subcultured HAC bound substantial amounts of \([^3H]\text{mepyramine}, which was displaced by unlabelled mepyramine, tripepallenamine, and chlorpheniramine at concentrations above 1 μmol/l (e.g., Fig. 2), but not by 2.5 mmol/l ranitidine or cimetidine. Preincubation of the HAC for 17 h with murine recombinant IL1 (12 units/ml) had no effect on the amount of \([^3H]\text{mepyramine} subsequently bound by the cells.\]

**Fig. 2** Displacement by chlorpheniramine of \([^3H]\text{mepyramine} bound to HAC. The curve shows the amount of \([^3H]\text{mepyramine} bound to HAC as a function of the amount of chlorpheniramine added. The \([^3H]\text{mepyramine} binding was performed as described in 'Materials and methods'.**

**Fig. 1** Effect of H₁ antagonists on the histamine stimulated PGE production by HAC. The chondrocytes were preincubated for 24 h with 20% (v/v) SF. The activating medium was removed and the cells were washed with HBSS before adding DMEM for one hour. ○ = DMEM; Δ = DMEM + histamine (17.8 μmol/l); ■ = DMEM + histamine (17.8 μmol/l) + mepyramine; □ = DMEM + histamine (17.8 μmol/l) + chlorpheniramine, △ = DMEM + histamine (17.8 μmol/l) + tripepallenamine. Results are means (SEM) (bars) of triplicate determinations.

**Effect of Histamine on HAC Intracellular Cyclic AMP**

Histamine (17.8 μmol/l) in the presence of a phosphodiesterase inhibitor produced an approximately fivefold increase in HAC intracellular cyclic AMP, a stimulation effectively inhibited by the two H₂ but not H₁ receptor antagonists (Table 3). The histamine induced rise in HAC cyclic AMP was not a consequence of increased PGE production as the inclusion of indomethacin (14 μmol/l) failed to prevent the increase. Furthermore, the chondrocytes used in the cyclic AMP studies had not been preincubated with SF and would have had negligible values for PGE biosynthesis, especially over such short (five minute) experimental incubations.
Table 3  Effect of H₁ and H₂ antagonists on the histamine induced increase of intracellular cyclic AMP in human articular chondrocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cyclic AMP (pmol/well)</th>
<th>Inhibition of cyclic AMP increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM+IBMX* (0-8 mmol/l)</td>
<td>4.23 (0-38)†</td>
<td></td>
</tr>
<tr>
<td>Histamine (17-8 μmol/l)</td>
<td>22.54 (0-28)</td>
<td></td>
</tr>
<tr>
<td>+ Mepyramine (12-3 μmol/l)</td>
<td>23-61 (1-27)</td>
<td>0</td>
</tr>
<tr>
<td>+ Tripelennamine (17-1 μmol/l)</td>
<td>23-03 (0-40)</td>
<td>0</td>
</tr>
<tr>
<td>+ Ranitidine (15-7 μmol/l)</td>
<td>5-71 (0-17)</td>
<td>92</td>
</tr>
<tr>
<td>+ Cimetidine (19-8 μmol/l)</td>
<td>6-71 (0-26)</td>
<td>87</td>
</tr>
</tbody>
</table>

Histamine and its antagonists were added to the cells for five minutes, after which the medium was removed and the cells assayed for cyclic AMP.
*IBMX = isobutyl-1-methylxanthine.
†Values are the mean (SEM), n=3.

Discussion

We have previously shown that histamine stimulates human articular chondrocytes to produce increased amounts of prostaglandin E₂, an event probably mediated via histamine H₁ receptors. This study has demonstrated the ability of three H₁ receptor antagonists to cause a dose related inhibition of histamine stimulated PGE₂ production at concentrations below 10⁻⁶ mol/l, an effect not shown by histamine H₂ receptor antagonists. Although much of the data have been obtained from ‘activated’ chondrocyte cultures, this resulting from a 20 hour preincubation with SF or IL1, a similar histamine stimulation of PGE₂ production was obtained with control, ‘non-activated’ chondrocytes. The possession of H₁ receptors therefore appears not to be dependent upon preincubation with SF or IL1, but is a feature of both control and ‘activated’ chondrocytes.

The existence of H₁ receptors on HAC was confirmed by the ³H labelled H₁ antagonist mepyramine, which bound readily and with specificity to all the HAC preparations tested. There was no evidence that IL1 pretreatment increased the expression of histamine H₁ receptors by the chondrocytes since no change in ³Hmepyramine binding was observed for cells incubated with or without IL1. Stimulation of the H₁ receptor in some tissues has previously been associated with an increase in intracellular cyclic guanosine-5’-monophosphate formation, but attempts to detect an increase in this nucleotide from histamine stimulated HAC were not satisfactory, and the PGE response proved to be a much better index for H₁ receptor activation.

Evidence for the existence of H₂ receptors on HAC was provided by the histamine induced rise in intracellular cyclic AMP recently reported. The association of the H₂ receptor with adenylate cyclase, and the production of cyclic AMP has been demonstrated in homogenates from many tissues, including cardiac muscle, gastric mucosa, and brain, and also in isolated cells such as adipocytes and certain white blood cells. Therefore the prevention of the histamine induced rise in HAC cyclic AMP by H₂ receptor antagonists (Table 3), together with the parallel shift of the histamine dose-response curve by increasing concentrations of cimetidine, provides conclusive evidence that chondrocytes express H₂ receptors.

This study presents evidence that HAC possess both H₁ and H₂ histamine receptors and that the histamine induced stimulation of cyclic AMP and prostaglandin production is mediated via two separate processes (H₁ and H₂ respectively). The possession of both receptor types on chondrocytes is not unique since rat glomerular mesangial cells and articular chondrocytes have recently been shown to respond to histamine by events mediated by both receptors. Further studies are required to assess whether these receptor induced processes are interactive. The histamine induced rises in PGE₂ and cyclic AMP may influence many cellular processes, including the production of degradative enzymes such as collagenase and plasminogen activator described for human synovial cells. In addition, recent studies have shown that the local production of PGE₂ transforms cultured fibroblasts to cells with dendritic morphology and increased proteinase expression.

The finding that both human articular chondrocytes and rheumatoid synovial cells have surface histamine receptors is an important observation in view of the recent reports of raised histamine levels found in rheumatoid but not osteoarthritic synovial fluids. Cells obtained from trabecular bone also responded to high concentrations of histamine with increased cyclic AMP, and 10⁻² - 10⁻³ M histamine was reported to reduce bone resorption in cultured mouse calvaria. The extent to which histamine may influence the metabolism and degradative mechanisms in joint disease is uncertain, but the recent reports of mast cells in synovial fluids from patients with diverse arthritides, the increased numbers of mast cells in rheumatoid synovial tissues, and the observation of mast cells at sites of cartilage erosion in rheumatoid joint specimens, suggest that the mast cell may be implicated in some of the pathophysiological changes observed in joint disease. Indeed, as chondrocytes are stimulated to produce collagenase by mast cell products and possess both histamine H₁ and H₂ receptors it is probable that histamine ‘activation’ could have profound effects on various aspects of chondrocyte biology.
metabolism, an interaction that may well be relevant to the aberrant chondrocytic behaviour observed in degenerative osteoarthritis and some examples of rheumatoid joint disease.

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References


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