

Histamine H₁ receptors on adherent rheumatoid synovial cells in culture: demonstration by radioligand binding and inhibition of histamine-stimulated prostaglandin E production by histamine H₁ antagonists

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SUMMARY Histamine H₁ receptors have been demonstrated on adherent rheumatoid synovial cells using biochemical and radioligand binding assays *in vitro*. The addition of histamine (17.8 µmol/l) to nine primary cultures of adherent rheumatoid synovial cells resulted in a two- to 21-fold increase in the production of prostaglandin E (PGE). This increase was inhibited by three H₁ receptor antagonists (mepyramine, tripeleennamine, and chlorpheniramine) in a dose related manner at concentrations below 10⁻⁶ mol/l. Competitive binding assays with [³H]mepyramine gave ED₅₀ values of approximately 10⁻⁵ mol/l for the three H₁ antagonists. H₂ receptor antagonists (cimetidine and ranitidine) did not inhibit the histamine induced increase in PGE and did not compete effectively with the binding of H₁ antagonists.

Rheumatoid synovial tissue produces high levels of prostaglandin E (PGE), which has been implicated in the pathophysiology of inflammatory joint disease.¹ The cellular interactions and factors involved in the increased production of PGE in the arthritic joint are poorly understood, but *in vitro* studies have shown that the mononuclear cell product interleukin 1 greatly increases PGE production by cultured rheumatoid synovial cells (RSC).² We have recently shown that histamine also increases PGE production from cultures of synovial fibroblasts and human articular chondrocytes, an observation especially pronounced after preincubation with interleukin 1.³ Since mast cells are reported to be present in increased numbers in rheumatoid synovium^{4,5} and increased histamine concentrations have been measured in rheumatoid compared with osteoarthritic synovial fluids⁶ it would seem that histamine has the potential to act *in vivo*.

The action of histamine on cells is mediated by at

least two distinct types of receptor,⁷ and our earlier studies have shown that the histamine stimulation of PGE production is mediated via the H₁ type.³ In the present study we have examined the susceptibility of this process to inhibition by various concentrations of three H₁ antagonists using primary cultures of RSC. We report here the affinity of these antagonists for the H₁ receptor and their ability to inhibit the histamine stimulation of prostaglandin E synthesis.

Materials and methods

Materials were obtained from the sources given by Taylor *et al.*⁸ with the addition of the following: [³H]PGE₂ and [³H]mepyramine were obtained from Amersham International, Amersham, Bucks, UK; anti-PGE serum was obtained from Miles Labs Ltd, Slough, UK; recombinant murine interleukin 1 (IL1) was a generous gift from Roche Products Ltd, Welwyn Garden City, Herts, UK. One unit of IL1 activity induces 50% maximal proliferative response in thymocyte costimulation assay.

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CELL CULTURE

Rheumatoid synovial tissue was obtained from

remedial synovectomies and knee arthroplasties of patients with classic rheumatoid arthritis. The tissue was dissociated enzymatically as described previously,⁹ and adherent synovial cell cultures were established in plastic wells of 16 mm diameter (24 wells/plate; Nunc, Denmark) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum (FCS) containing penicillin, streptomycin, and amphotericin. These adherent cell cultures represented a mixed population classified morphologically as fibroblastic, dendritic, and macrophagic¹⁰ and were used for experiments within seven days. The cells were cultured and maintained during the experiments at 37°C in a water saturated atmosphere at 5% (v/v) CO₂ in air. The cells were washed three times with Hanks's balanced salt solution before adding the treatments. After one or two hours the culture media were removed and stored at -20°C for prostaglandin E (PGE) assay. Total cell protein, prepared by a 6% (w/v) perchloric acid precipitate of the cells, was measured by the method of Lowry *et al.*¹¹

PROSTAGLANDIN E STUDIES

PGE was measured in culture media by radioimmunoassay¹² using an antiserum with similar specificity towards the prostaglandins E₁ and E₂ and charcoal coated with dextran to separate bound from free.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 vortex mixing 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 DP 5500 curve fit processor. The sensitivity of the assay was 5 pg when determined by two standard deviations at zero dose.

RADIOLIGAND BINDING STUDIES

Radioligand binding assays were performed on rheumatoid synovial cell monolayers derived from seven specimens. To obtain sufficient cells for repeated measurement subcultures were established with conventional trypsin treatment and replating at reduced density. One consequence of using subcultured cells (from two weeks to several months) was that they assumed a uniform fibroblastic morphology. The binding experiments were performed on confluent monolayers in plastic wells of 35 mm diameter (six wells/plate; Costar, USA) at a temperature of 4°C. The multiwell plate was supported on a porous, stainless steel platform covered with a film

of ice water in an ice bath. Growth medium was removed and the cells were washed with 3 ml of ice cold binding buffer (125 mM triethanolamine, 25 mM MgCl₂, and 5 mM histidine at pH 7.0) and allowed to equilibrate in this buffer for 30 min on ice. Binding experiments were initiated by replacing with 0.9 ml of the same buffer containing [³H]mepyramine (10 nmol/l) with or without histamine antagonist. After 90 min the medium was removed and the cells were rapidly rinsed three times with a total of 6 ml of ice cold binding buffer. The cells were detached by incubation at 37°C with 0.1% (w/v) trypsin in Hanks's balanced salt solution and were quantitatively transferred to vials for liquid scintillation counting. Initial binding experiments confirmed that at 4°C [³H]mepyramine binding reached equilibrium by one hour and was unchanged during the following three hours. The proportion of radioligand bound by cells which received only the [³H]mepyramine was between 20% and 30% of the total (10⁵ cpm) antagonist added. All treatments were performed in duplicate or triplicate and cpm bound differed by less than 10%.

Although the total cell protein content per well showed variation with different cell cultures (between 179 µg and 322 µg protein/well), within a single experiment the variation in cell protein/culture well was consistently less than 5%.

Results

HISTAMINE EFFECTS ON PGE PRODUCTION

Histamine (17.5 µmol/l) caused an increase in PGE production when added to nine different preparations of primary RSC cultures. This represented a two- to 21-fold increase of control values, but the magnitude of the response was larger for those cell cultures with a low level of PGE production (Table 1). The histamine stimulation of PGE production was prevented by the H₁ receptor antagonist mepyramine, whereas a much higher concentration of the H₂ antagonist cimetidine had little inhibitory action (Table 2). Further evidence that this PGE response induced by histamine is mediated by a receptor of the H₁ type was obtained using lower concentrations of mepyramine and two other H₁ antagonists, chlorpheniramine and tripeleminamine. These agents all reduced the histamine stimulated PGE production at concentrations below 10⁻⁶ mol/l in a dose related manner (Fig. 1). This experiment was repeated with five different RSC cultures and the results obtained suggested the antagonists all had a similar potency. The highest concentration of each H₁ antagonist used in the experiments, when added without histamine, did not significantly change basal PGE production by the RSC.

Table 1 Effect of histamine on PGE production by primary cultures of adherent RSC

RSC sample	PGE (ng/h/mg protein)		
	Control (DMEM)	Histamine (17.8 μmol/l)	Fold increase
399	15.08 (0.62)	85.66 (6.95)	5.7
400	4.25 (0.34)	49.02 (3.82)	11.5
405	23.16 (1.77)	88.05 (4.76)	3.8
406	48.62 (1.96)	100.67 (2.84)	2.1
409	18.61 (0.99)	41.50 (0.92)	2.2
410	17.26 (1.54)	53.76 (3.16)	3.1
411	18.32 (3.72)	74.53 (3.35)	4.1
421	32.12 (2.31)	69.05 (2.82)	2.2
429	4.08 (0.51)	86.30 (2.52)	21.2

RSC were grown for three to seven days. The culture medium was removed and the cells were washed as described in the 'Materials and methods' section before adding DMEM for one to two hours. Results are mean (SEM) for triplicate or quadruplicate determinations. The PGE production stimulated by histamine was greater than control at $p < 0.005$ for all samples.

Table 2 Effect of H_1 and H_2 antagonists on histamine stimulated PGE production by a primary culture of adherent RSC

Treatment	PGE (ng/h/mg protein)
DMEM	4.25 (0.34)
Histamine (17.8 μmol/l)	49.02 (3.82)
Histamine (17.8 μmol/l)+mepyramine (1.3 μmol/l)	10.11 (1.04)*
Histamine (17.8 μmol/l)+cimetidine (40 μmol/l)	41.30 (5.01)
Mepyramine (1.3 μmol/l)	4.04 (0.44)
Cimetidine (40 μmol/l)	2.91 (0.34)

RSC were grown for seven days. The culture medium was removed and the cells were washed as described in the 'Materials and methods' section before adding DMEM for one hour. Results are mean (SEM) for quadruplicate determinations. * $p < 0.0005$ when compared with histamine alone.

$[^3H]$ MEPYRAMINE BINDING TO RSC

Seven preparations of subcultured RSC bound substantial amounts of $[^3H]$ mepyramine, which at an antagonist concentration below 10^{-3} mol/l was only displaced by unlabelled mepyramine and two other H_1 antagonists. In contrast, a human melanoma cell line (DX3-azaC-LT5.1)¹⁴ under identical culture and experimental conditions bound only a fraction of the radioligand bound by the RSC (Fig. 2). Higher concentrations of $[^3H]$ mepyramine resulted in increased binding to the cells, and this showed no signs of being saturated at 200 nmol/l. The binding of $[^3H]$ mepyramine to RSC was reversible, less than 5% of that originally bound being left after a 10 min

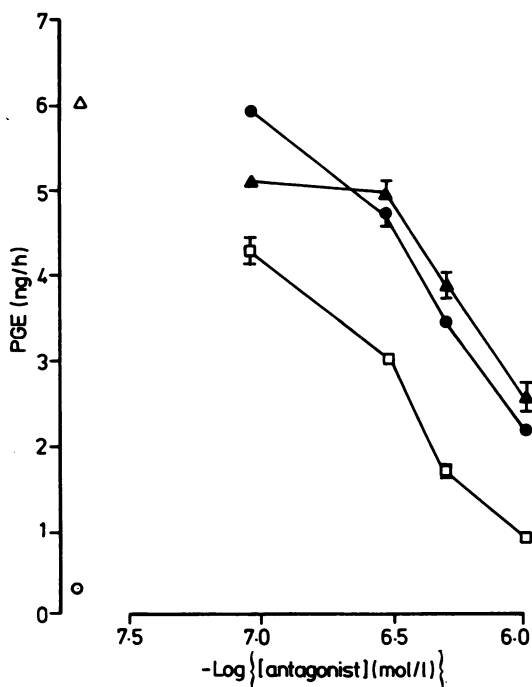


Fig. 1 Effect of H_1 antagonists on the histamine stimulated PGE production by primary rheumatoid synovial cell cultures. Primary cultures of RSC were grown for seven days. The culture medium was removed and cells were washed as described in the 'Materials and methods' section before adding DMEM for 90 min. ○=DMEM; △=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)+tripeleennamine. Results are mean (SEM) (bars) for triplicate determinations.

incubation with 3 mM unlabelled mepyramine (result not shown). Preincubation of the RSC for 20 h with murine recombinant IL1 (12 units/ml) or human interferon- γ (10 units/ml) had no effect on the amount of $[^3H]$ mepyramine subsequently bound by the cells, suggesting that 'activation' of rheumatoid synovial fibroblasts did not result in an increased expression of H_1 receptors (results not shown).

SPECIFICITY (DRUG COMPETITION) STUDIES

To measure the relative binding affinity of different histamine receptor antagonists ED_{50} values were calculated from competitive binding curves using $[^3H]$ mepyramine. The ED_{50} value is the dose of unlabelled ligand yielding 50% displacement of the labelled ligand $[^3H]$ mepyramine and was calculated using the polygonal program on a Beckman DP 5500 processor. Fig. 3 is an example of the type of

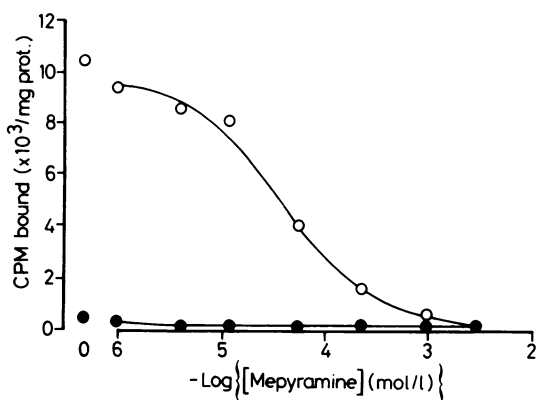


Fig. 2 Displacement by unlabelled mepyramine of [^3H]mepyramine bound to rheumatoid synovial fibroblasts and a human melanoma cell line. The curve shows the amount of [^3H]mepyramine bound by the two cell types as a function of the amount of unlabelled mepyramine added. The [^3H]mepyramine binding was performed as described in the 'Materials and methods' section, using the same tracer preparation for both the RSC (\circ) and the human melanoma cell line (\bullet).

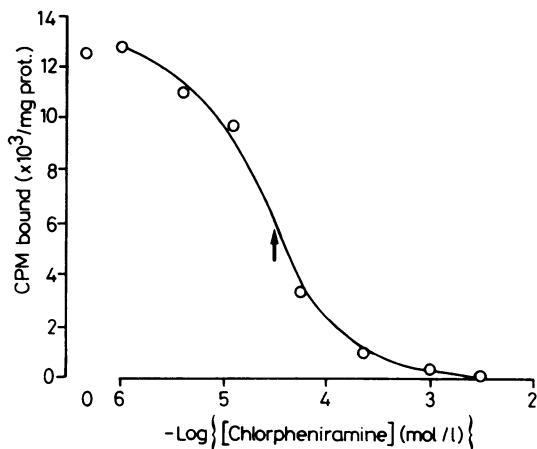


Fig. 3 Displacement by chlorpheniramine of [^3H]mepyramine bound to rheumatoid synovial fibroblasts. The curve shows the amount of [^3H]mepyramine bound by the synovial fibroblasts as a function of the amount of chlorpheniramine added. The [^3H]mepyramine binding was performed as described in the 'Materials and methods' section. The arrow shows the ED_{50} value obtained for this experiment.

curve obtained and it shows the displacement of [^3H]mepyramine binding by the H_1 antagonist chlorpheniramine. Of the various agents tested, only the H_1 antagonists had ED_{50} values below 10^{-4} mol/l (Table 3). In contrast, histamine and the H_2

Table 3 ED_{50} values for competition of [^3H]mepyramine binding to rheumatoid synovial fibroblasts

Agent	Action	ED_{50} ($\mu\text{mol/l}$)
Mepyramine	H_1 antagonist	25.3 (4.5)
Tripeleennamine	H_1 antagonist	28.3 (4.7)
Chlorpheniramine	H_1 antagonist	29.7 (1.6)
Histamine	H_1, H_2 agonist	>10 000
Ranitidine	H_2 antagonist	>10 000
Cimetidine	H_2 antagonist	>10 000

The binding of [^3H]mepyramine and the ED_{50} calculation were performed as described in the experimental section. The values are mean (SEM) of determinations performed on three different RSC preparations for each antagonist.

antagonists ranitidine and cimetidine had negligible effects, and histamine even at 10^{-2} mol/l failed to produce a 50% reduction in binding.

Discussion

The addition of histamine to primary cultures of adherent rheumatoid synovial cells significantly increased the rate of PGE production. The variations in magnitude of the histamine response were mainly dependent upon differences in control PGE values rather than those obtained after histamine exposure (Table 1). The variations in control PGE values for each of the nine RSC cultures may have several explanations, for example, the different proportions of fibroblasts and macrophages, the time elapsed after initial plating, and the extent of 'activation' by non-adherent cells before washing, as well as the availability of the prostaglandin substrate arachidonic acid. An important contribution by the latter was recently reported for the histamine stimulated PGE production by subcultured synovial fibroblasts.³ Moreover, as arachidonic acid is released from endogenous phospholipids by phospholipase A_2 , an enzyme activated by Ca^{++} , it is of interest that histamine acting via H_1 receptors has recently been reported to increase free Ca^{++} in endothelial cells.¹⁵

The involvement of H_1 receptors in the present study was shown by using specific histamine H_1 antagonists. Whereas mepyramine, chlorpheniramine, and tripeleennamine all reduced the histamine-stimulated PGE production at concentrations below 10^{-6} mol/l in a dose related manner, a 40-fold higher concentration of the H_2 antagonist cimetidine had no effect. Moreover, specific binding of H_1 antagonists to the cells was demonstrated with [^3H]mepyramine, which bound to all the RSC preparations tested. The bound radioligand was

displaced preferentially by other H₁ antagonists, but not by histamine or the H₂ antagonists cimetidine and ranitidine even at very high concentrations (10⁻² mol/l). The human melanoma cell line DX3-azaC-LT5.1,¹⁴ cultured under identical experimental conditions, failed to bind [³H]mepyramine, an observation that essentially excludes the possibility of non-specific binding of [³H]mepyramine to RSC.

Specific binding of [³H]mepyramine to histamine H₁ receptors has previously been demonstrated in homogenates of guinea pig brain¹⁶ and muscle,¹⁷ and also for human mononuclear cells¹⁸ and rat cardiomyocytes.¹⁹ The similar ED₅₀ value obtained from competitive binding experiments for the three H₁ antagonists corresponds with their similar potency for preventing the histamine-stimulated PGE production by RSC. The ED₅₀ values calculated for chlorpheniramine and mepyramine in this study are similar to those reported for the characterisation of histamine H₁ receptors on human lung.²⁰ Indeed, several classes of H₁ antagonist-binding sites have been reported in human lung, some with low affinity for [³H]mepyramine having a dissociation constant around 10⁻⁴ M,²⁰ an observation that may explain the failure to obtain binding saturation with 200 nM [³H]mepyramine in the present study.

The monocyte-macrophage product interleukin 1 (IL1) has a number of stimulatory effects on cultured RSC which include increased prostaglandin E and collagenase production,¹ enhanced hyaluronate synthesis,²¹ and even morphological changes.²² Exposure of RSC to murine IL1 (at concentrations which increased both PGE and collagenase production) did not affect [³H]mepyramine binding. A similar observation was made with the lymphokine interferon-γ which has been found to increase class II antigen expression and inhibit collagen synthesis by RSC in culture.²³ Despite the failure of IL1 and interferon-γ to increase H₁ receptor expression, the former was found to amplify the histamine stimulated PGE response of synovial fibroblasts,³ an observation possibly explained by the reports that histamine increases the availability of arachidonic acid in RSC³ and that protein synthesis stimulated by IL1 is essential for enhanced prostaglandin expression.²⁴

Conventional treatments for inflammatory joint disease usually attempt to control prostaglandin production by the use of non-steroidal anti-inflammatory drugs. This report shows that mixed primary RSC cultures, and specifically rheumatoid synovial fibroblasts, possess histamine H₁ receptors which when stimulated produce increased amounts of PGE. Human articular chondrocytes also possess H₁ receptors (unpublished data), and an increased distribution of mast cells has been reported in

rheumatoid synovium^{4,5} and at sites of cartilage erosion.²⁵ With recent reports of raised histamine levels in rheumatoid synovial fluids⁶ it may be an opportune time to reconsider the potential use of both histamine H₁ and H₂ antagonists in the management of inflammatory joint disease.²⁶

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