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Histamine H_2 receptors on foetal-bovine articular chondrocytes

David J. TAYLOR, Joan R. YOFFE and David E. WOOLLEY
University Department of Medicine, University Hospital of South Manchester, Manchester M20 8LR, U.K.

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The dose–response curve of histamine-induced cyclic AMP elevation in monolayer cultures of primary foetal-bovine articular chondrocytes was displaced to the right by cimetidine. In addition, H_2 but not H_1 antagonists prevented the histamine-induced cyclic AMP elevation, suggesting histamine activates chondrocyte adenylate cyclase through an H_2 receptor.

The effect of histamine on cells is mediated via at least two distinct receptors. Histamine-induced contraction of smooth muscle from various organs such as gut and bronchi is competitively blocked by the classical antihistamines such as diphenhydramine, mepyramine or tripelennamine (Ash & Schild, 1966). The receptors involved in such actions have been classified as H_1 receptors. Histamine stimulation of gastric acid secretion, relaxation of rat uterus and stimulation of the guinea-pig heart are not antagonized by mepyramine and related compounds but are blocked by histamine H_2 receptor-blocking agents such as burimamide, cimetidine and metiamide (Black *et al.*, 1972). H_2 receptors are invariably associated with adenylate cyclase and stimulation of the receptor has consistently resulted in increased tissue levels of cyclic AMP (for review, see Johnson, 1982).

Our recent studies have been designed to assess the role of the mast cell in the pathophysiology of the rheumatoid joint. H_2 receptors have been reported in the synovial vasculature of dogs and histamine-stimulation produced a consistent vasodilator effect (Grennan *et al.*, 1975). Moreover, histamine increased the vascular permeability of rabbit synovium via H_2 receptors (Al-Haboubi & Zeitlin, 1980). As we have preliminary evidence that mast cells are found at sites of cartilage erosion in rheumatoid joints (Bromley *et al.*, 1983) we decided to examine chondrocytes with respect to H_2 receptor physiology. The present paper provides the first evidence that chondrocytes have histamine H_2 receptors.

Experimental

Materials

Dulbecco's modified Eagle's medium with glutamine/Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], fungizone and heat-inactivated foetal-calf serum were from Grand Island Biological Company. Benzyl penicillin, streptomycin sulphate and ranitidine were from Glaxo, Ware, Herts., U.K. Histamine dihydrochloride, theophylline, mepyramine maleate and cyclic AMP were purchased from Sigma, Poole, Dorset, U.K. Cimetidine was a gift from Smith, Kline and French, Welwyn, Herts., U.K., and tiotidine was a gift from Pharmaceuticals Division of Imperial Chemical Industries PLC. Chlorpheniramine maleate was from Allen and Hanburys, London, U.K. Adenosine 3′:5′-cyclic phosphoric acid, 2′-*O*-succinyl 3-[^{125}I]iodotyrosine methyl ester and [U-^{14}C]proline were obtained from Amersham International, Amersham, Bucks., U.K. Rabbit anti-(cyclic AMP–bovine serum albumin) serum was from Miles Laboratoreis, Stoke Poges, Bucks., U.K. Beef heart phosphodiesterase was purchased from Boehringer, Lewes, Sussex, U.K.

Cultures

Chondrocytes were obtained by proteolytic digestion of articular cartilage (Gibson *et al.*, 1982) derived from the hip, knee and ankle joints of foetal calves. The cells were grown in six-well plastic cluster dishes (3.5cm diameter) in Dulbecco's modified Eagle's medium (DMEM) with 10% (v/v) foetal-calf serum supplemented with penicillin, streptomycin and fungizone. The cultures were incubated at 37°C in CO_2/air (1:19) in a water-saturated atmosphere. When the cells were confluent (after 5–6 days) the medium was removed and replaced with DMEM + 10% acid-treated foetal-calf serum containing 2.4mM-theophylline and the agent(s) under investigation. The cells were incubated at 37°C for 5min, after which the medium was removed and the cells precipitated with 6% (w/v) $HClO_4$. Initial studies showed that the maximal rise in chondrocyte cyclic AMP was achieved within 5min of adding histamine.

Chondrocytes *in vitro* tend to assume a fibroblastoid morphology with concomitant switch from the predominant synthesis of type II to type I collagen (Mayne *et al.*, 1976; Von der Mark *et al.*, 1977). The chondrocytes used in these studies were examined for collagen synthesis using fluorograms of [U-^{14}C]proline-labelled collagens as described by Gibson *et al.* (1982). Primary and secondary cultures produced predominantly type II collagen, which confirmed the chondrocytic nature of the cells. Viability of the cells upon exposure to the H_2 and H_1 antagonists was shown to be good as judged by measurement of glucose uptake after extended incubations.

Intracellular cyclic AMP assay

The precipitated cells in $HClO_4$ were scraped off the well and transferred to a ground-glass homogenizer; the well was washed once with 0.5ml of water, which was added to the homogenizer. After thorough homogenization the preparation was transferred to a centrifuge tube and the homogenizer was rinsed with a further 0.5ml of water, which was added to the centrifuge tube. After centrifugation at 1500*g* for 5min the supernatant was removed and the protein was stored at −20°C for protein estimation (Lowry *et al.*, 1951). Of the supernatant 1ml was neutralized with 30% (w/v) $KHCO_3$ saturated with KCl. After standing the tube on ice for 30min, the precipitate was removed by centrifugation as described above. To 0.5ml of the neutralized supernatant was added 1.0ml of water and this was assayed for cyclic AMP by radioimmunoassay based on the method of Chiang & Kowalski (1982). The assay was found to be sufficiently sensitive without an acetylation step (0.03pmol as determined by 2 standard deviations at zero cyclic AMP dose) and a sample volume of 200μl in a total incubation volume of 500μl was used. Another modification was the use of 1.0ml of a pre-incubated mixture of polyethylene glycol/human plasma as the separation system. This reagent was prepared by adding 20ml of human plasma to 180ml of 18% (w/v) polyethylene glycol 6000 and, after mixing, centrifuging at 2000*g* for 15min at 4°C. The superantant was discarded and the precipitate resuspended in 200ml of the polyethylene glycol solution. After adding 1.0ml of the reagent to all the assay tubes they were vortex-mixed and centrifuged at 2000*g* for 15min at 4°C. The supernatants were decanted and the radioactivity of the pellets counted in a gamma counter that was connected to a Beckman DP5500 curve-fit processor. A 1h incubation of six cyclic AMP samples at 25°C with phosphodiesterase caused a reduction in the detectable cyclic AMP of greater than 85% in all the samples.

Results and discussion

Monolayer cultures of foetal-bovine articular chondrocytes produced increased levels of intracellular cyclic AMP in response to histamine exposure. The cyclic AMP response to increasing concentrations of histamine is shown in Fig. 1. The H_2 antagonist cimetidine displaced this dose–response curve to the right in a concentration-dependent manner (Fig. 1). The approximately parallel response curves suggest that histamine interacts with chondrocyte adenylate cyclase through an H_2 receptor. Further support for this is provided by the H_2 antagonists ranitidine and tiotidine, which were more effective than cimetidine in eliminating the histamine-induced rise in cyclic AMP (Table 1). In contrast, the H_1 antagonists mepyramine and chlorpheniramine failed to prevent the histamine-induced elevation in cyclic AMP over the same concentration range (Table 1).

The order of potency of the H_2 antagonists, as suggested by the lower concentrations of each agent used in Table 1, resembles that found for their ability to inhibit gastric acid output in the dog (Cavanagh *et al.*, 1980).

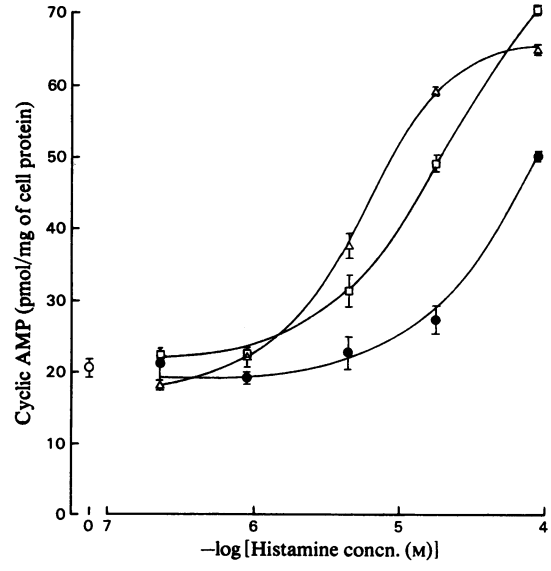

Fig. 1. *Effect of cimetidine on the histamine dose–response curve of intracellular cyclic AMP in foetal-bovine articular chondrocytes*

Primary chondrocyte cultures were grown to confluence. The culture medium was replaced with that described in the Experimental section, incubated for 5min and the cells assayed for cyclic AMP. ○, Control culture medium; △, culture medium + histamine; □, culture medium + histamine + 0.8μM-cimetidine; ●, culture medium + histamine + 7.9μM-cimetidine. Results are means ± S.E.M. (bars) for triplicate determinations.

Table 1. *Effect of H_1 and H_2 antagonists on the histamine-induced increase of intracellular cyclic AMP in foetal-bovine articular chondrocytes*

Chondrocytes at first passage were grown to confluence. The culture medium was replaced with that described in the Experimental section, incubated for 5 min and the cells assayed for cyclic AMP. Results are mean values ± S.E.M. for triplicate determinations.

Treatment	Cyclic AMP (pmol/mg of cell protein)	Inhibition of cyclic AMP increase (%)
Control	17.03 ± 1.81	
Histamine (17.8 μM)	94.59 ± 2.00	
Histamine (17.8 μM) + chlorpheniramine (5.1 μM)	89.53 ± 2.69	7
Histamine (17.8 μM) + chlorpheniramine (25.6 μM)	85.04 ± 1.97	12
Histamine (17.8 μM) + mepyramine (5.0 μM)	68.08 ± 1.12	34
Histamine (17.8 μM) + mepyramine (24.9 μM)	74.37 ± 4.48	26
Histamine (17.8 μM) + cimetidine (4.0 μM)	35.98 ± 0.72	76
Histamine (17.8 μM) + cimetidine (19.8 μM)	19.81 ± 1.17	96
Histamine (17.8 μM) + ranitidine (3.2 μM)	21.01 ± 1.31	95
Histamine (17.8 μM) + ranitidine (15.7 μM)	12.31 ± 0.10	100
Histamine (17.8 μM) + tiotidine (3.1 μM)	11.57 ± 0.65	100
Histamine (17.8 μM) + tiotidine (15.6 μM)	10.90 ± 0.22	100

Malemud *et al.* (1982) reported that exposure of rabbit articular chondrocytes to prostaglandin E_2 resulted in increased levels of total cyclic AMP. However, unlike the results reported here for histamine, their observations were due to released cyclic AMP rather than elevated cellular levels. Houston *et al.* (1982) have recently reported that prostaglandins, adrenaline and isoprenaline significantly stimulate adenylate cyclase activity in passaged cultures of human articular chondrocytes. Thus it appears that chondrocytes are responsive to a variety of factors, although the phenotypic expression to each has yet to be studied in detail.

The effect of cell passage on the sensitivity of foetal-bovine chondrocytes to histamine was also studied. Primary chondrocyte cultures were established and subcultured at confluence up to the third passage. Each of the primary and passaged cultures was treated in an identical manner with histamine concentrations, and the cyclic AMP response is shown in Fig. 2. The maximal response to histamine increased with passage, especially between the primary and P1 cells. This change in histamine responsiveness with cell passage may be related to the other changes in phenotypic expression reported for chondrocytes maintained *in vitro* (Mayne *et al.*, 1976; Benya *et al.*, 1978; Gibson *et al.*, 1982).

Other possible explanations are that the target cells are able to respond to changes in ambient ligand concentration by regulating the number of their surface receptors (see Catt *et al.*, 1979), by improved coupling between the respective components or by an increase in the total available adenylate cyclase. There is no evidence that receptor affinity increases with passage since the con-

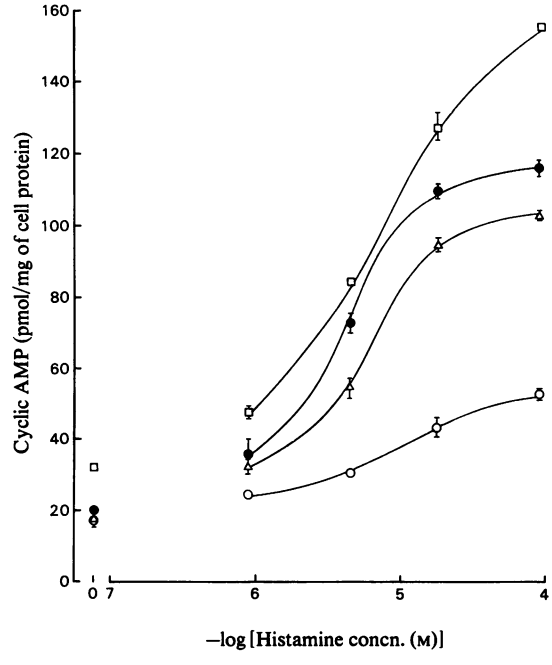

Fig. 2. *Effect of cell passage on cyclic AMP response to histamine in foetal-bovine articular chondrocytes*

Responses in primary (○) culture and after the first (△), second (□) and third (●) passage are shown. Experimental procedures were as described in the Experimental section. The primary culture and first, second and third passages represent 6, 14, 20 and 27 days respectively. All measurements were carried out on confluent cultures. Results are means ± S.E.M. (bars) for triplicate determinations.

centration of histamine required to produce a 50% response remains constant.

One of the factors that has limited the understanding of the histamine receptor/cyclic AMP system has been the availability of a suitable experimental cell line. The observation that cultures of foetal-bovine articular chondrocytes possess histamine H_2 receptors suggests that these cells might provide such a model system. The significance of these findings in relation to cartilage destruction in rheumatoid arthritis and osteo-arthritis is, as yet, unexplored.

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