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Histamine H_2 receptors on chondrocytes derived from human, canine and bovine articular cartilage

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Histamine (1–100 μM) induced a concentration-dependent increase in intracellular cyclic AMP in monolayer cultures of human, canine and foetal-bovine articular chondrocytes. The dose–response curve for histamine in each culture was progressively displaced to the right with increasing concentrations of cimetidine, an H_2-receptor antagonist. The histamine-induced cyclic AMP elevation in human articular chondrocytes was also significantly decreased by ranitidine, another H_2 antagonist, but not by the H_1 antagonists mepyramine and chlorpheniramine. These findings indicate that histamine activates chondrocyte adenylate cyclase through an H_2 receptor. The cyclic AMP response of human chondrocytes to histamine was many times greater than that measured for synovial fibroblasts under similar conditions. Such findings suggest that mast-cell–chondrocyte interactions *in vivo* may contribute to changed chondrocyte metabolism in joint disease.

The concept of two distinct types of pharmacological receptors (H_1 and H_2) for histamine actions is now well established (Black *et al.*, 1972). The histamine-induced contraction of smooth muscle from gut and bronchi is a result of H_1-receptor stimulation, possibly mediated by intracellular cyclic GMP (Johnson, 1982). Histamine H_2 receptors have been identified in gastric mucosa, rat uterus and guinea-pig heart; their stimulation is associated with adenylate cyclase activation and increased tissue levels of cyclic AMP (for review, see Johnson, 1982). Mast cells and basophils are the major source of histamine in the body, and their interaction with various tissues and histamine-sensitive cells has been implicated as a contributory factor in the pathophysiology of several allergic and inflammatory disorders (Lewis & Austen, 1981; Wasserman, 1979).

We recently identified histamine H_2 receptors on foetal-bovine articular chondrocytes (Taylor *et al.*, 1983), but the applicability of this finding with regard to mature chondrocytes from animals of other species was uncertain. Here we present evidence that histamine H_2 receptors occur not only on foetal-bovine, but also on mature canine and human articular, chondrocytes.

Experimental

Materials were obtained from the sources given by Taylor *et al.* (1983), with the addition of the following: cyclic [8-^{3}H]AMP was obtained from Amersham International, Amersham, Bucks., U.K. 3-Isobutyl-L-methylxanthine and prostaglandin E_2 were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. Cimetidine was a gift from Smith, Kline and French, Welwyn Garden City, Herts., U.K.

Culture

Chondrocytes were obtained by proteolytic digestion of articular cartilage derived from the hip, knee and ankle joints of foetal calves and from the femoral condyles of mature dogs by the method of Gibson *et al.* (1982). The human articular chondrocytes were obtained by proteolytic digestion of macroscopically normal articular cartilage from femoral heads obtained from remedial surgery, as described by Meats *et al.* (1980). The foetal-bovine and canine chondrocytes were grown in plastic cluster dishes in Dulbecco's modified Eagle's medium with 10% (v/v) foetal-calf serum supplemented with penicillin, streptomycin and fungizone. The human chondrocytes were grown in plastic cluster dishes with 15%-(v/v)-foetal-calf-serum supplement.

Cultures were incubated at 37°C in CO_2/air (1:19) in a water-saturated atmosphere. When the cells were confluent, the medium was removed and replaced with medium containing 800 μM-isobutyl-L-methylxanthine and the agent(s) under investi-

gation. The cells were incubated at 37°C for 5min, after which the medium was removed and the cells precipitated with 6% (w/v) $HClO_4$, or with boiling water for the human chondrocytes.

Synovial fibroblasts were prepared from human rheumatoid synovial pannus by proteolytic digestion and cultured as previously described by Dayer *et al.* (1976).

Intracellular cyclic AMP assay

The precipitated cells were scraped off the well and homogenized (Taylor *et al.*, 1983). After centrifugation at 1500*g* for 5min, the supernatant was removed and the protein pellet dissolved in 0.1M-NaOH before protein determination (Lowry *et al.*, 1951). The supernatant obtained from the cells precipitated with boiling water was assayed for cyclic AMP without further treatment, whereas those precipitated with $HClO_4$ were neutralized and diluted as described previously (Taylor *et al.*, 1983). Comparative studies showed that both precipitating treatments were equally effective. The cyclic AMP was measured either by radioimmunoassay (Taylor *et al.*, 1983) or by a competitive protein-binding assay (Tsang *et al.*, 1972). In the latter the protein binder was isolated from bovine adrenal glands and diluted in assay buffer [50mM-Tris/HCl/4mM-EDTA/0.5% (w/v) bovine serum albumin/2mM-theophylline/0.05% (w/v) NaN_3, pH7.5]. 100μl was then added to 200μl of sample and 100μl of cyclic [8-^{3}H]AMP. After 4h at 0°C, 5mg of dextran-coated charcoal was added to each tube, followed by centrifugation at 1500*g* for 10min at 4°C. The supernatant was decanted and counted for radioactivity in a liquid-scintillation spectrometer connected to a Beckman DP5500 curve-fit processor. The sensitivity of the assay was 0.04pmol (determined by 2S.D. at zero cyclic AMP dose), and cross-reactivity of the binder was 0.18% for cyclic GMP and not detectable at 5mM for AMP, ADP and ATP.

Results and discussion

Monolayer cultures of canine and human articular chondrocytes produced increased levels of intracellular cyclic AMP in response to histamine, as previously observed for foetal-bovine articular chondrocytes (Figs. 1*a*, 1*b* and 1*c*). The approximately parallel displacement of the dose–response curve produced by the H_2 antagonist cimetidine suggests that adenylate cyclase stimulation is mediated by a histamine H_2 receptor. The response curves are similar for chondrocytes from all three species, with significant stimulation above controls present at 1μM-histamine and maximal stimulation at approx. 100μM. A second H_2 antagonist, ranitidine, also prevented the histamine-induced rise in cyclic AMP in human chondrocytes. In contrast, the H_1 antagonists chlorpheniramine and mepyramine had no significant effect (Table 1), indicating that this chondrocyte histamine receptor is of the H_2 type.

Dose ratios based on the EC_{50} (histamine concentration that produces 50% of maximal cyclic AMP response) values of histamine in the absence and presence of different concentrations of cimetidine were calculated for the human chondrocytes. A Schild plot (Arunlakshana & Schild, 1959) was constructed by plotting log(dose ratio − 1) against the logarithm of the molar concentrations of cimetidine and fitting the points by least-squares linear regression (Fig. 1*c*). The calculated pA_2 (negative logarithm of the concentration required to shift the response curve by a factor of 2) value of cimetidine against histamine-induced cyclic AMP accumulation in human chondrocytes was 6.3, which is close to those (6.10 and 6.22) reported for other H_2-receptor-containing systems such as guinea-pig ventricle and hippocampus (Johnson, 1982). The slope of the Schild plot for cimetidine against histamine was 0.89, which is sufficiently close to unity to suggest that the antagonism is competitive.

Chondrocytes maintained *in vitro* as monolayer cultures tend to assume a fibroblastoid morphology (Von der Mark *et al.*, 1977). This was not apparent in the canine and bovine chondrocytes, which were in culture no more than 6 days. However, the human chondrocytes did assume a fibroblastoid morphology, probably as a result of the longer culture period (27–38 days) required to obtain sufficient cells. The human chondrocyte response to histamine was therefore compared with that of subcultured human synovial fibroblasts. Fig. 2 demonstrates that synovial fibroblasts exposed to histamine produced only a fraction of the increase in cyclic AMP observed in the human chondrocytes. Moreover, the latter were more sensitive to histamine, with significant stimulation occurring at 1μM, whereas only the highest histamine concentration tested produced a significant increase in synovial fibroblast cyclic AMP. Thus, despite their similar morphological appearance, the two cell types differ markedly in the magnitude of their response to exogenous histamine. Interestingly, even the small increase in cyclic AMP observed in the synovial fibroblasts could be prevented by a histamine H_2, but not H_1, antagonist (Fig. 2). Primary cultures of human articular chondrocytes cultured for 12 days responded to histamine (163μM) by an increase in intracellular cyclic AMP from 19.63 to 104.61pmol/mg of protein. This result is similar to that obtained for primary cultures of bovine and canine chondrocytes (Figs. 1*a* and 1*b*) and indicates that the

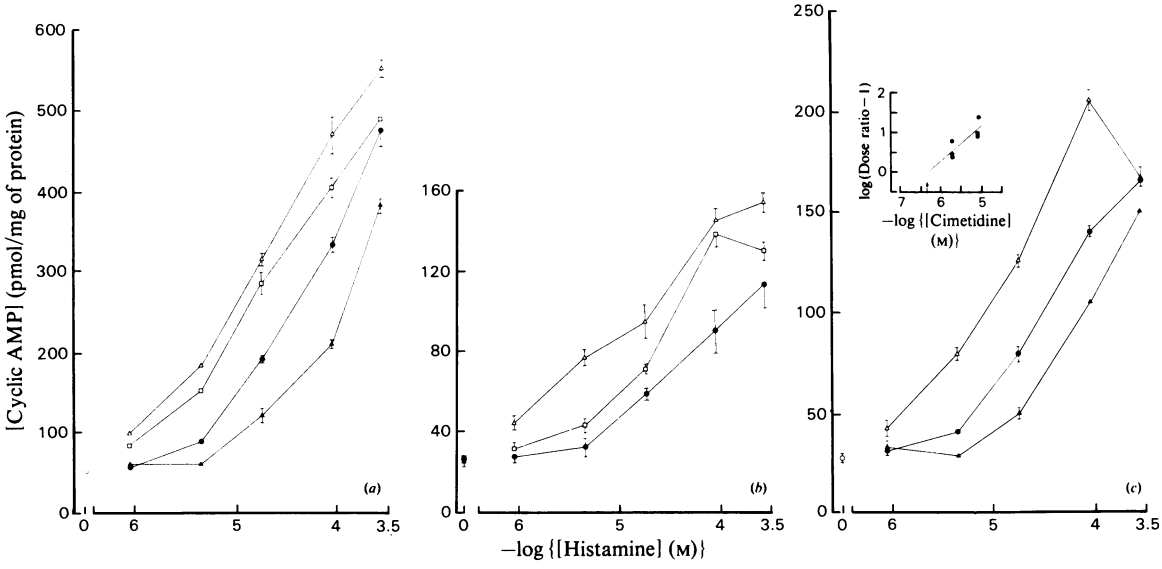

Fig. 1. *Effect of cimetidine on the histamine dose–response curve of intracellular cyclic AMP in (a) foetal-bovine, (b) canine and (c) human articular chondrocytes*

(*a*) Primary chondrocyte cultures 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. ○, Control medium; △, culture medium + histamine; □, culture medium + histamine + 0.4 μM-cimetidine; ●, culture medium + histamine + 1.9 μM-cimetidine; ▲, culture medium + histamine + 7.9 μM-cimetidine, Results are means ± S.E.M. (bars) for triplicate determinations. (*b*) Primary chondrocytes were cultured and treated as described in (*a*). △, Culture medium + histamine; □, culture medium + histamine + 1.9 μM-cimetidine; ●, culture medium + histamine + 7.9 μM-cimetidine. Results are means ± S.E.M. (bars) for quadruplicate determinations. (*c*) Subcultured chondrocytes were cultured and treated as described in (*a*). ○, Control culture medium; △, culture medium + histamine; ●, culture medium + histamine + 1.9 μM-cimetidine; ▲, culture medium + histamine + 7.9 μM-cimetidine. Results are means ± S.E.M. (bars) for triplicate determinations. The inset depicts the Schild plot of cimetidine concentration against histamine-induced cyclic AMP accumulation obtained by least-squares regression of log(dose ratio − 1) versus log(molar concn. of cimetidine). Dose ratios were based on six concentration–response curves for histamine-induced cyclic AMP accumulation in the presence of two concentrations of cimetidine (1.9 and 7.9 μM); each concentration–response curve was obtained from 15 determinations. The slope was 0.89 and the intersection with the abscissa gave the pA_2 (see the text) value of 6.3 (arrow).

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

Human chondrocytes 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	Concn.	[Cyclic AMP] (pmol/mg of protein)	Inhibition of cyclic AMP increase (%)
Control		34.18 ± 2.71	
Histamine (10.9 μM)		159.64 ± 2.89	
+ Cimetidine	4.0 μM	91.30 ± 5.77	55
	19.8 μM	48.29 ± 3.50	89
+ Ranitidine	3.2 μM	58.81 ± 1.38	82
	15.9 μM	46.98 ± 1.43	90
+ Chlorpheniramine	5.1 μM	168.85 ± 2.27	0
	25.6 μM	158.77 ± 2.68	1
+ Mepyramine	5.0 μM	157.11 ± 7.14	2
	24.9 μM	147.70 ± 7.50	10

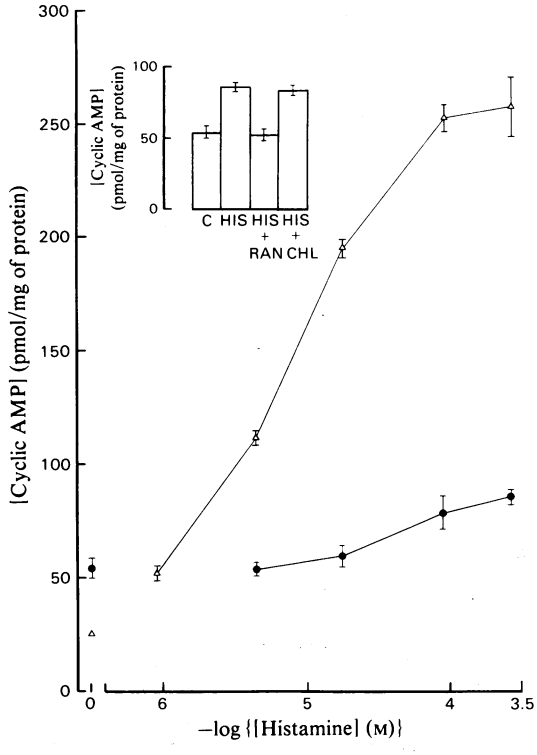

Fig. 2. *Effect of histamine on intracellular cyclic AMP in human articular chondrocytes and synovial fibroblasts*
Subcultured chondrocytes and synovial fibroblasts were grown to confluence. The culture medium was replaced with that described in the Experimental section, incubated for 5 min, and the cells assayed for cyclic AMP. Results are mean values ± S.E.M. (bars) for triplicate determinations. △, Articular chondrocytes; ●, synovial fibroblasts. The inset shows the effects of 15.7 μM-ranitidine (RAN) and 25.6 μM-chlorpheniramine (CHL) on cyclic AMP accumulation elicited by 268 μM-histamine (HIS) in synovial fibroblasts. 'C' indicates control. Each histogram represents the mean ± S.E.M. (bars) of triplicate determinations.

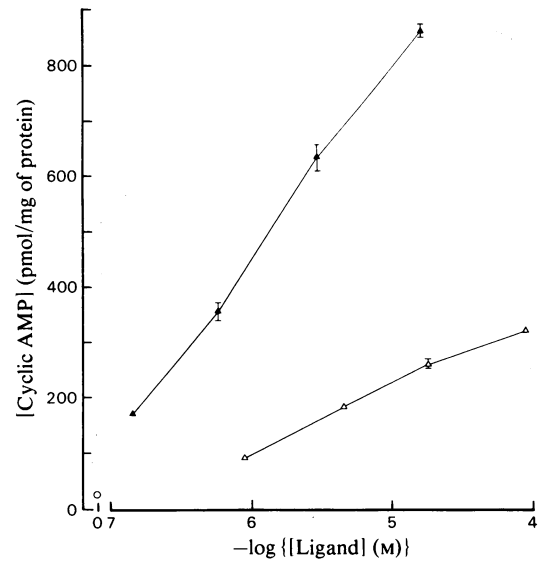

Fig. 3. *Effect of histamine and prostaglandin E_2 on intracellular cyclic AMP in foetal-bovine articular chondrocytes*
Primary chondrocyte cultures were grown to confluence. The culture medium was replaced with that described in the Experimental section, incubated for 5 min, and the cells assayed for cyclic AMP. ○, Control culture medium; △, culture medium + histamine; ▲, culture medium + prostaglandin E_2. Results are means ± S.E.M. (bars) for triplicate determinations.

histamine response of human chondrocytes is not associated with the phenotypic and morphological changes that are known to occur with prolonged culture (Mayne *et al.*, 1976; Benya *et al.*, 1978).

As prostaglandin E_2 was reported to stimulate cyclic AMP biosynthesis in rabbit articular chondrocytes (Malemud *et al.*, 1982) and stimulated human chondrocyte adenylate cyclase (Houston *et al.*, 1982), the effect of histamine and prostaglandin E_2 on intracellular cyclic AMP was compared in primary foetal-bovine articular chondrocytes. Both agents produced a severalfold increase in chondrocyte cyclic AMP, with prostaglandin E_2 producing a larger overall increase and acting at lower concentrations than histamine (Fig. 3). Further evidence that both histamine and prostaglandin E_2 could have a physiological effect on chondrocyte cyclic AMP was demonstrated by their ability to increase cyclic AMP in secondary cultures of foetal-bovine chondrocytes in the absence of a phosphodiesterase inhibitor. Under these conditions the cyclic AMP reached maximal levels within 2 min of adding the agents and returned to baseline values after 20 min (D. J. Taylor & D. Plowman, unpublished work).

The significance of the histamine H_2 receptor on human articular chondrocytes is uncertain, but it may have relevance to some pathophysiological changes observed in joint disease. Histamine is released *in vivo* from mast cells and basophils, and an increased incidence of mast cells has been reported in rheumatoid synovium (Graziano *et al.*, 1983; Crisp *et al.*, 1983). Of greater significance to the present study are the observations of mast cells at sites of cartilage erosion in rheumatoid joints (Bromley *et al.*, 1984), and the presence of measurable quantities of histamine in the synovial fluids of rheumatoid and osteoarthrotic joints (Igari, 1977). Thus, as both histamine and prostaglandin E_2 are known to be present in

diseased joints (Robinson & Levine, 1974), it seems possible that they could be responsible for the 'activation' of chondrocytes. Subsequent changes in cyclic AMP levels may have profound effects on aspects of chondrocyte metabolism, such as prostaglandin and/or proteinase production, as well as collagen and proteoglycan biosynthesis, but these effects have yet to be examined. In any event, mast cell–chondrocyte interactions mediated by histamine release may explain the suppression of acute and chronic inflammation by the H_2-receptor antagonist cimetidine in adjuvant arthritic rats (Al-Haboubi & Zeitlin, 1982).

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