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Histamine H_2 receptors mediate the inhibitory effect of histamine on human eosinophil degranulation

*,[1]Charles I. Ezeamuzie & [1]Elizabeth Philips

[1]Department of Pharmacology and Toxicology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat, Kuwait

1 The effect of histamine on human eosinophil degranulation and the receptor mediating such effect were studied *in vitro* using the complement C5a-mediated eosinophil peroxidase (EPO) release model.

2 Following pre-treatment with 5 μg ml^{-1} cytochalasin B(CB), C5a induced a concentration-dependent release of EPO from eosinophils isolated from healthy donors.

3 Histamine (0.1–50 μM), but not L-histidine, inhibited concentration-dependently C5a-induced EPO release with IC_{50} (95% CI) of 0.6 μM (0.3–1.2 μM) and maximal inhibition of $\approx$60%.

4 A similar effect was seen with the selective H_2 agonists dimaprit (IC_{50} (95% CI) = 6.9 μM (3.2–10.6 μM)) and amthamine (IC_{50} (95% CI) = 0.4 μM (0.2–0.7 μM)). Neither the selective H_1 agonist 6-(2-(4-imidazolyl)ethylamino)-N-(4-trifluoromethylphenyl) heptanecarboxamide(HTMT), nor the selective H_3 agonists imetit (up to 100 μM) had any significant effect.

5 The inhibition by histamine was reversed by cimetidine (0.1–30 μM) and other H_2 antagonists, but not the H_1 antagonist mepyramine (1.0–100 μM), nor the H_3 antagonist thioperamide (1.0–100 μM). Cimetidine (1–30 μM) shifted to the right the dimaprit log dose-response curve, producing a pA_2 value of 5.9 and Schild's plot slope of 0.98, thus confirming simple competitive antagonism.

6 Histamine (10–100 μM) increased intracellular level of adenosine 3′,5′-cyclic monophosphate, which was completely abolished by cimetidine (30 μM), but not mepyramine or thioperamide. The cyclic AMP analogue – dibutyryl cyclic AMP – also inhibited degranulation (IC_{50} $\sim$300 μM). The cyclic AMP phosphodiesterase(PDE) IV inhibitor rolipram (10 μM) synergistically enhanced the inhibition of EPO release by histamine.

7 These results suggest that histamine, *via* stimulation of H_2 receptors and a consequent elevation of intracellular levels of cyclic AMP, inhibits human eosinophil degranulation.
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Keywords: Histamine; H_2 receptors; eosinophils; degranulation; adenylate cyclase

Abbreviations: CB, cytochalasin B; CI, confidence interval; DMSO, dimethylsulphoxide; EPO, eosinophil peroxidase; FMLP, N-formyl methionyl-leucyl-phenylalanine; HTMT, 6-[2-(4-imidazolyl)ethylamino]-N-(4-trifluoromethylphenyl)-heptanecarboxamide;PDE IV, adenosine 3′,5′-cyclic monophosphate phosphodiesterase, type IV

Introduction

Eosinophils are known to play important roles in the pathophysiology of allergic diseases, especially asthma (Frigas & Gleich 1986; Barnes 1989). They contain several granular cationic proteins, including eosinophil peroxidase (EPO) and eosinophil cationic protein, which when secreted by the infiltrating bronchial eosinophils cause airway epithelial damage, resulting in the development of bronchial hyperreactivity that is characteristic of asthma (Laitinen *et al*., 1985; Motijima *et al*., 1989).

It has been known for many years that the activity of eosinophils may be modulated by mediators released by degranulating mast cells, especially histamine (Clark *et al*., 1977; Anwar & Kay 1980; Pincus *et al*., 1982, Plaut & Lichtenstein 1982). Histamine is a ubiquitous mediator whose effects are mediated *via* at least three distinct receptors – H_1, H_2, and H_3 (Ash & Schild 1966; Black *et al*., 1972; Arrang *et al*., 1983). While the presence of H_1 and H_2 receptors on eosinophils has become well established (Clark *et al*., 1977; Wadee *et al*., 1980; Sedgwick & Busse 1990), that of H_3 is still equivocal (Raible *et al*., 1992; 1994). In general, the effect of histamine on granulocytes is complex, consisting of both stimulation and inhibition. The H_1 receptors are generally stimulatory, and on both guinea-pig and human eosinophils, mediate the stimulation of superoxide ions generation (Pincus *et al*., 1982) enhancement of chemokinesis (Clark *et al*., 1977; Wadee *et al*., 1980) and the increased expression of C3b receptors (Anwar & Kay 1980). The H_2 receptors, on the other hand, are generally inhibitory – with such inhibition being mediated *via* the activation of membrane adenylate cyclase and a consequent increase in the intracellular levels of cyclic AMP (Wadee *et al*., 1980; Sedgwick & Busse 1990). More recently, histamine-induced increase in intracellular (Ca^{2+}) in human eosinophils has been reported, but this effect seems to be mediated by what appears to be atypical H_3 receptors, since it was blocked by selective H_3 antagonists (although with atypical rank order of potency), but not the H_1 or H_2 antagonists (Raible *et al*., 1992; 1994).

Few studies have addressed the modulatory effect of histamine on granulocyte degranulation. In human basophils, histamine was reported to perform autoregulatory functions – inhibiting degranulation and hence its own release – *via* membrane H_2 receptors (Lichtenstein & Gillespie 1975; Tedeschi *et al*., 1991). In human neutrophils, there is conflicting evidence in the ability of histamine, through H_2 receptors, to inhibit lysosomal enzyme release (Busse &

*Author for correspondence; E-mail: ezeamuzie@hsc.kuniv.edu.kw

Sosman 1976; Marone *et al*., 1980). Surprisingly, no studies, to our knowledge, have examined the effect of histamine on eosinophil degranulation, in spite of the fact that both eosinophil and mast cell degranulation (hence histamine release) generally occur in the same microenvironment of allergic reactions. With the realization of the crucial roles that eosinophils play in allergic reactions, as well as the availability of new improved methods for the isolation of large numbers of pure eosinophils, the need for such studies seem obvious.

The aim of this study was, therefore, to determine if histamine modulates human eosinophil degranulation and, if so, to characterize the receptor(s) involved.

Methods

Isolation of blood eosinophils

Fresh blood was obtained from consenting healthy adults who have taken no medications in the last 72 h. Eosinophils were purified by a slight modification of the immunomagnetic method (Hansel *et al*., 1991). Briefly, three parts of sodium citrate-anti-coagulated (13 mM final) blood was mixed with one part of 1% (w v^{-1} of 0.9% saline) hydrated methylcellulose solution to sediment the erythrocytes over 30 min at room temperature. The leucocyte-rich supernatant was collected and centrifuged at $200 \times g$ for 10 min at room temperature. After aspirating off the platelet-rich supernatant, the pelleted leucocytes were washed in 'wash buffer' (Ca^{2+}- and Mg^{2+}-free, 10 mM HEPES-buffered Hanks balanced salt solution containing 0.25% bovine serum albumin (BSA)) and resuspended in the same buffer at approximately 10% of the original blood volume. A 2-ml aliquot was then layered on a 2-step percoll gradient (1.080 and 1.090 g ml^{-1}) and centrifuged at $900 \times g$ on Beckman (GS-6R) centrifuge for 20 min at room temperature. The upper layers (mononuclear cells and percoll) were discarded and the pellet (granulocytes) was recovered and washed twice in the same buffer by centrifugation at $600 \times g$ for 10 min at 4°C. After a hypotonic lysis of contaminating erythrocytes with ice-cold distilled water, and readjusting the tonicity with the same volume of double strength saline, the cells were washed, counted and resuspended at a concentration of 2×10^7 cells ml^{-1} in the wash buffer. For the eosinophil purification, 1.25 ml of the granulocyte suspension was then mixed with 5 μl mouse anti-human CD16 monoclonal antibody in a siliconized test tube and incubated on ice for 1 h with frequent gentle rotation. Cells were then washed twice in wash buffer and after the final wash, the cells were pellet by centrifugation and then resuspended in 500 μl of prewashed immunomagnetic beads pre-coated with affinity purified sheep anti-mouse IgG (2×10^8 coated beads) and incubated in ice for 1 h with frequent tube rotation. The immunomagnetically-labelled neutrophils were removed by magnetic extraction. The purified eosinophils were then recovered by centrifugation and resuspended in reaction buffer ('wash buffer' containing 2 mM Ca^{2+} and 1 mM Mg^{2+}) for experiments. The eosinophil purity was assessed by differential count of a Wright-Giemsa stained cytosmear. The final cell preparation routinely consisted of over 98% pure eosinophils. Viability was determined by trypan blue exclusion and always exceeded 98%.

Eosinophil peroxidase (EPO) release

Purified eosinophils were used at a concentration of 5×10^5 cells ml^{-1}. Fifty microlitres of prewarmed cell suspension containing 2.5×10^4 cells was dispensed into each well of a microplate. Then, 100 μl of the reaction buffer containing 10 μg ml^{-1} cytochalasin B (CB) was added and after 10 min pre-incubation, the cells were stimulated with 50 μl of human recombinant C5a. The mixture was further incubated for 30 min at 37°C. It had been determined in pilot experiments that this time was sufficient for the virtual completion of the degranulation process. At the end of the incubation period, reaction was stopped by cooling on ice and after centrifugation at $600 \times g$, for 10 min, 50 μl aliquots of the supernatant as well as Triton X-100-lysed cells (for total content determination) were taken for the determination of the released enzymes. EPO activity was measured by the O-phenylenediamine (OPD) method as previously reported (Kroegel *et al*., 1989). Briefly, OPD substrate solution containing 0.4 mg ml^{-1} OPD and 0.4 mg ml^{-1} urea hydrogen peroxide in PBS-citrate buffer (pH 4.5) was prepared from SIGMA FAST® OPD tablets. One hundred microlitres of this substrate was added to 50 μl of the samples in a microplate and incubated for 30 min at 37°C. After incubation, the reaction was then stopped with 50 μl of 4 M H_2SO_4 and the plate read at 490 nm. Values were expressed as percentage of total content, using the amount obtained in half the same number of cells, after lysis, as 50%. The recovery of released EPO activity was usually above 80% at the end of 30 min incubation, but usually lower with more prolonged incubation.

Cyclic AMP measurements

One million purified eosinophils, resuspended in 150 μl of BSA-free reaction buffer containing 30 μM rolipram (when indicated) were dispensed into each well of the 96-well plate and incubated for 10 min at 37°C. Reaction was then started by the addition of 50 μl of warmed histamine or other stimuli. Three minutes later – a time previously determined to be best for this response) – the reaction was stopped by the direct addition of 22.2 μl of IN HCl. After thorough mixing and further incubation for 10 min, the plate was centrifuged at $1500 \times g$ for 10 min and 200 μl of the supernatant taken and stored at – 43°C pending cyclic AMP assay.

Cyclic AMP levels were measured, after acetylation, using commercially available ELISA kit, and following manufacturer's instructions. The sensitivity of the assay was 0.01 pmoles $well^{-1}$.

Chemicals and biochemical reagents

The following reagents and materials were purchased from Sigma Chemical Co., St. Louis, U.S.A.: recombinant human C5a, FMLP, percoll, (N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulphonic acid))(HEPES), bovine serum albumin (BSA), OPD, dimethylsulphoxide (DMSO), histamine dihydrochloride, dibutyryl cyclic AMP, L-histidine, cytochalasin B and all inorganic salts (Sigma-ultra). Items obtained from Tocris Cookson Ltd, Bristol, U.K., include dimaprit dihydrochloride, thioperamide maleate, toitidine, imetit dihydrobromide, cimetidine, amthamine dihydrochloride, HTMT dimaleate and R(−)-α-methylhistamine. Ranitidine and rolipram were obtained from Research Biochemicals Inc., Natick, MA, U.S.A. Mouse monoclonal anti-human CD16 antibody (clone FcR gran1) was obtained from CLB, Amsterdam, The Netherlands, while the magnetic beads (coated with sheep anti-mouse IgG) were supplied by Dynal AS, Oslo, Norway. The cyclic AMP kit (direct method) was obtained from Assay Designs Inc, Ann Arbor, Michigan, U.S.A.

Stock solutions of toitidine and rolipram were made in DMSO to concentrations in the range ($1-4 \times 10^{-1}$ M) and

then diluted directly in buffer. The final concentration of DMSO present at the highest drug concentrations did not exceed 0.05% – a concentration that has no effect on eosinophil responses. All the other drugs and reagents were first dissolved in distilled water and diluted down in reaction buffer.

Statistical analysis

Experimental data are presented as mean ± standard deviation from the number (n) of independent experiments. The IC_{50} values were calculated from the concentration-effect curves by non-linear regression analysis using GraphPad InPlot (GraphPad Software Inc., Philadelphia, U.S.A.). The pA_2 value was determined according to Arunlakshana & Schild (1959), while the pK_B values were determined using single concentrations of the antagonists and employing the formula $pK_B = -\log$ {[antagonist]/dose ratio-1}. Statistical significance (P) was determined by the unpaired t-test and analysis of variance (ANOVA) as appropriate (InStat, GraphPad, Software Inc. U.S.A.).

Results

C5a-induced eosinophil degranulation

In the presence of CB (5 μg ml^{-1}), C5a induced a concentration-dependent release of granular EPO from purified human eosinophils (Figure 1). The release generally began at around 10^{-9} M, and at the highest concentration tested, 10^{-7} M, a net EPO release of 43.8 ± 4.4% of the cell content was obtained. No EPO release occurred in the absence of CB. The submaximal concentration of C5a (10^{-8} M) was chosen for the subsequent experiments.

Effect of histamine and histamine receptor agonists on EPO release

Pre-incubation of eosinophils with histamine for 10 min before stimulation caused a pronounced and concentration-dependent inhibition of C5a-induced EPO release, Figure 2. The IC_{50} (95% CI) was 0.6 μM (0.3 – 1.2 μM) and maximal inhibition of 60.1 ± 5.2% at 50 μM. At concentrations up to 100 μM, no significant inhibition was seen with L-histidine, a direct precursor of histamine. Similar results were also obtained when EPO release was induced by N-formyl-methionyl-leucyl-phenylalanine (FMLP) in the presence of CB (data not shown).

To assess the histamine receptor(s) mediating the inhibition, the effect of selective agonists at the three histamine receptors was examined. As shown in Figure 3, both of the selective H_2 receptor agonists – dimaprit and amthamine (Eriks *et al.*, 1992), produced a concentration-dependent inhibition of EPO release, with IC_{50} values (95% CI) of 6.9 μM (3.2 – 10.6 μM) and 0.4 μM (0.2 – 0.7 μM), respectively. Maximal inhibitions (at the highest concentration tested, 100 μM) were 86.6 ± 7.4% and 58.4 ± 6.7% for dimaprit and amthamine, respectively. Thus, histamine and amthamine seem to have similar potency and efficacy, while dimaprit has a lower potency but near-maximal efficacy. In contrast, neither HTMT – the potent and selective H_1 agonist (Khan *et al.*, 1987) – nor the two selective H_3 agonists – imetit and R(−)-α-methylhistamine – produced any significant inhibition at concentrations up to 100 μM.

At the concentrations tested, none of these agonists had any significant effect on cell viability, peroxidase assay or recovery.

Effect of histamine receptor antagonists

The effects of selective antagonists at the three histamine receptors on histamine-induced inhibition of EPO release are shown in Figure 4. Pre-incubation of the cells with the standard H_2 receptor antagonist cimetidine (0.3 – 30 μM) for 5 min before the addition of histamine, resulted in concentration-dependent reversal of the inhibition, with almost complete reversal at 30 μM. A similar treatment with the selective H_1 antagonist mepyramine or the selective H_3 antagonist thioperamide failed to reverse the histamine-induced inhibition.

To further characterize the H_2 receptors, the nature of the receptor interaction was examined. As shown in Figure 5, cimetidine produced a concentration-dependent rightwards parallel shift of the log concentration-response curve for dimaprit (Figure 5a). The Schild's plot yielded a pA_2 value of 5.9 (95% CI = 5.7 – 6.2) and a slope of 0.98 (95% CI = 0.96 –

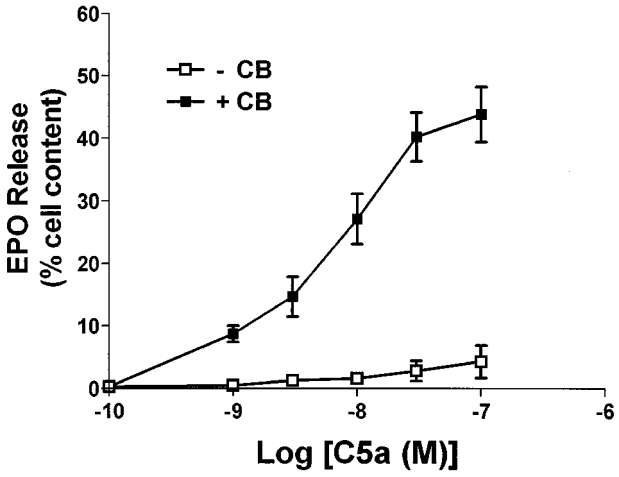

Figure 1 Concentration-effect relationship of C5a-induced release of EPO from human eosinophils in the presence and absence of 5 μg ml^{-1} cytochalasin B (CB). Values are means ± s.d. $n = 12$ (with CB) and $n = 4$ (without CB).

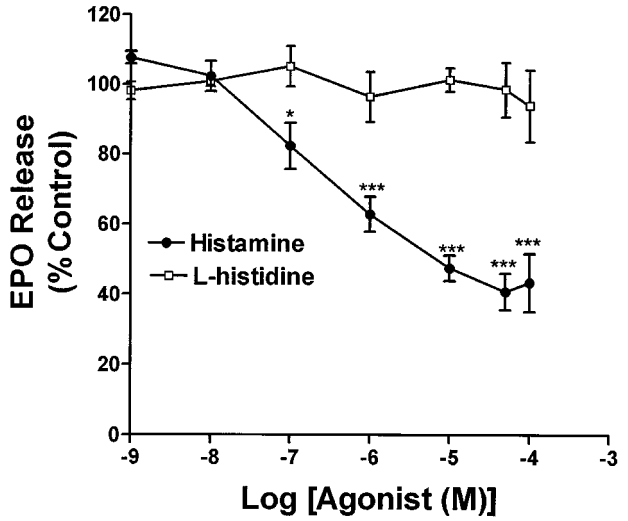

Figure 2 The effect of histamine and L-histidine on EPO release from human eosinophils induced by 10^{-8} M C5a in the presence of 5 μg ml^{-1} CB. Uninhibited release was in the range 15 – 38% of the cell content. Values are means ± s.d. $n = 11$ (histamine) and $n = 4$ (L-histidine). $^*P < 0.05$; $^{***}P < 0.001$.

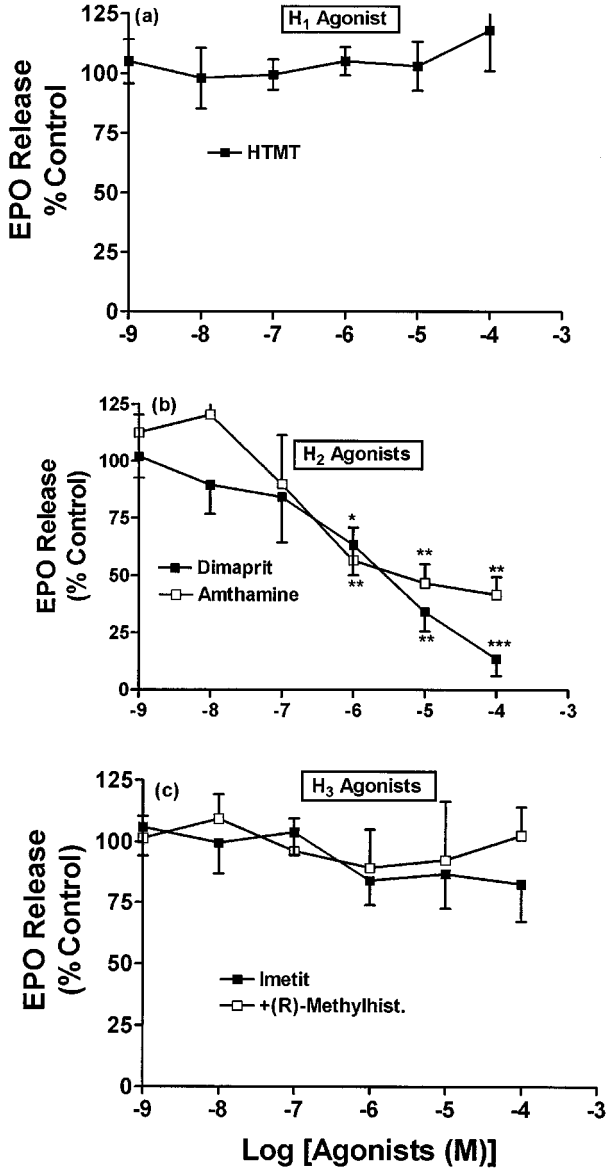

Figure 3 Effect of the various histamine receptor agonists on EPO release from human eosinophils induced by 10^{-8} M C5a in the presence of 5 μg ml^{-1} CB. Uninhibited release was in the range 15–38% of the cell content. Values are means ± s.d. for 5–7 experiments. *$P<0.05$; **$P<0.01$; ***$P<0.001$. HTMT = 6-[2-(4-imidazolyl)ethylamino]-N-(4-trifluoromethylphenyl)heptanecarboxamide).

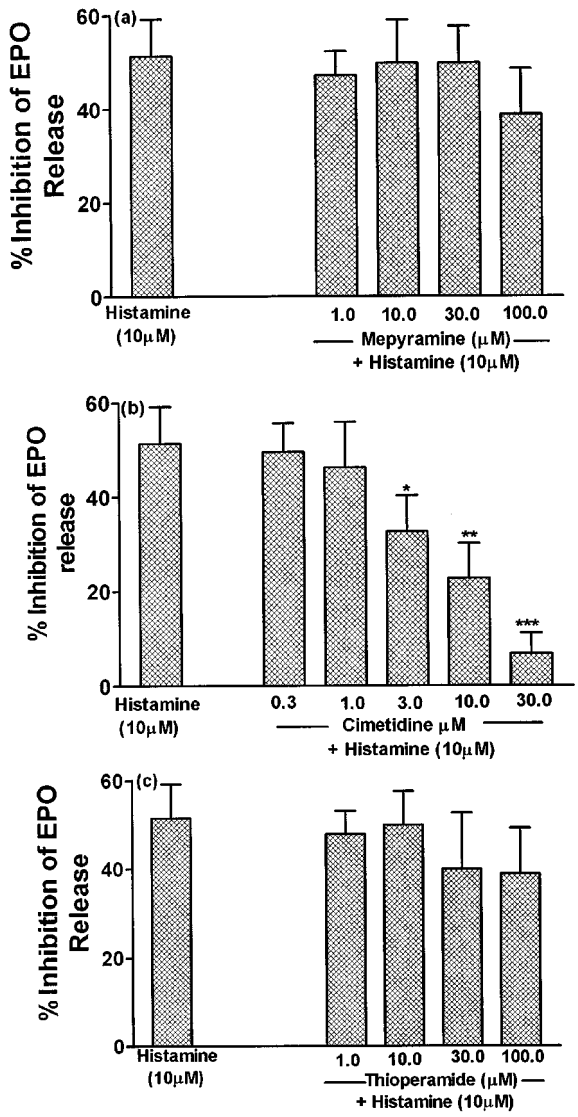

Figure 4 Reversal of histamine-induced inhibition of EPO release by H_2 receptor antagonist – cimetidine (b), but not the H_1 receptor antagonist - mepyramine (a) or the H_3 receptor antagonist – thioperamide (c). Cells were first incubated with the antagonist for 5 min, followed by histamine for a further 10 min before being stimulated with 10^{-8} M C5a in the presence of 5 μg ml^{-1} CB. The uninhibited releases were in the range 15–38% of cell content. Values are means ± s.d., $n=5$ *$P<0.05$; **$P<0.01$, ***$P<0.001$.

1.10), $n=4$, which is not significantly different from unity (Figure 5b), and thus indicating simple competitive antagonism. A similar result was also obtained using cimetidine vs histamine ($pA_2=5.8$ (95% CI = 5.6–6.1), and slope of 0.96 (95% CI = 0.94–0.99), $n=3$). Furthermore, antagonist potency comparison using single concentrations of cimetidine (3 μM), ranitidine (3 μM) and toitidine (0.3 μM), against histamine, yielded pK_B values of 5.8, 6.1 and 7.7, respectively. None of the antagonists had any direct EPO-releasing effect of their own or affected EPO assay or recovery.

Effect of histamine and dimaprit on intracellular cyclic AMP levels

In order to investigate the possible mechanism of H_2-mediated inhibitory action, the effect of histamine and dimaprit on the intracellular levels of cyclic AMP was studied. Following a 10 min pre-incubation of the cells with rolipram (30 μM), both agonists caused a concentration-dependent stimulation of cyclic AMP formation (Figure 6a). The mean content of cyclic AMP in unstimulated cells was 0.26 ± 0.07 pmoles 10^6 cells^{-1}, which was increased to 0.45 ± 0.21 pmoles 10^6 cells^{-1} in the presence of rolipram alone, $P>0.05$, $n=4$. At 10, 30 and 100 μM, histamine increased the levels to 0.98 ± 0.36, 1.72 ± 0.36 and 1.67 ± 0.25 pmoles 10^6 cells^{-1}, respectively. The increases produced by 30 μM and 100 μM were statistically significant ($P<0.05$ and <0.01, respectively), $n=4$. Dimaprit produced a similar, but less potent effect. Both drugs did not induce significant increases in cyclic AMP in the absence of rolipram. The cyclic AMP response produced by histamine (30 μM) was completely abolished by cimetidine, (30 μM) but not by a combination of mepyramine and thioperamide (10 μM each), (Figure 6b). The antagonists themselves did not affect cyclic AMP levels.

Effect of agents that increase or mimic intracellular cyclic AMP

The analysis of the effect of rolipram – a PDE IV inhibitor, and dibutyryl cyclic AMP – a cell-permeable analogue of cyclic AMP, is shown in Table 1. Rolipram (1 and 10 μM) failed to inhibit EPO release and caused only a small, statistically non-significant, increase in cyclic AMP, whereas histamine (1 μM) significantly inhibited EPO release, ($P<0.01$) but caused only a small increase in cyclic AMP. However, the combination of histamine (1 μM) and rolipram appeared to be synergistic for EPO release: $39\pm6.4\%$ for histamine (1 μM) alone, $2.7\pm1.5\%$ for rolipram (10 μM) alone and $64.2\pm5.1\%$ for the combination, $P<0.05$, $n=5$. For the cyclic AMP response, the combination was essentially additive (0.30 ± 0.12 pmoles 10^6 cells^{-1} for histamine (1 μM) alone,

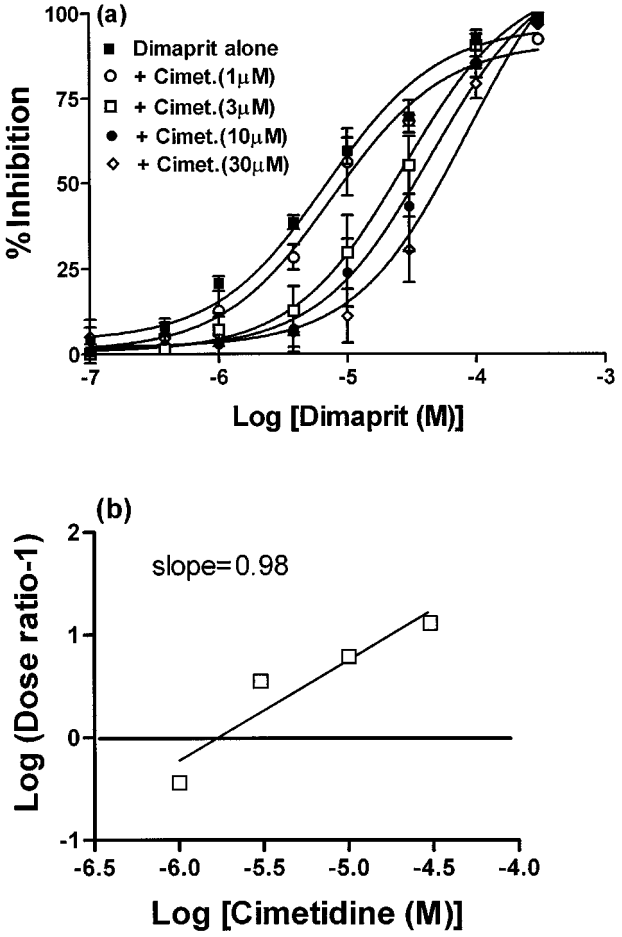

Figure 5 Antagonism by cimetidine of dimaprit-induced inhibition of C5a (10^{-8} M)-stimulated EPO release in the presence of CB (5 μg ml^{-1}). (a): dimaprit log-concentration-response curves for increasing concentrations of cimetidine ($n=4$). The uninhibited releases were in the range 15–38% of total cell content. Curves were fitted by non-linear regression analysis, (b): Schild's plot of the data in panel (a), which yielded a pA_2 of 5.9 (95% CI: 5.7–6.2) and a slope of 0.98(95% CI: 0.96–1.10).

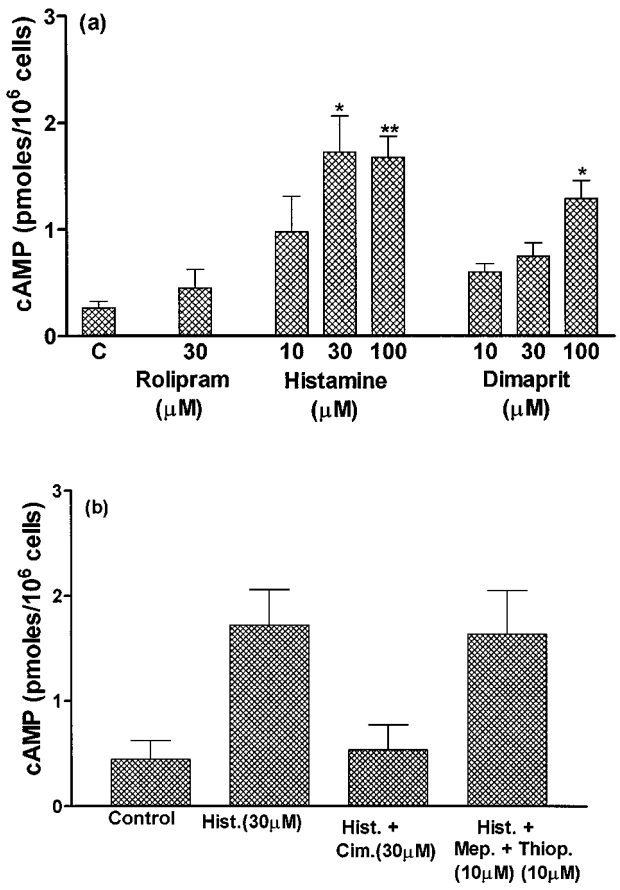

Figure 6 Histamine- and dimaprit-induced stimulation of intracellular cyclic AMP production in human eosinophils; (a): concentration-response relationship for both drugs; (b): inhibition by cimetidine, but not thioperamide and mepyramine, of the histamine-stimulated increase. Cells were pre-incubated with rolipram (and where applicable the receptor antagonists) for 10 min before being stimulated with histamine or dimaprit. The antagonists themselves had no significant effect on cyclic AMP levels. Values are means$\pm$s.d., $n=4$ for both panels. *$P<0.05$; **$P<0.01$ (compared with rolipram alone).

Table 1 Effect of rolipram and dibutyryl cyclic AMP on EPO release and intracellular cyclic AMP: interaction with histamine

	Mean % inhibition of EPO release[a] ($n=5$)	*Intracellular concentration of cyclicAMP* (pmoles 10^6 cells^{-1}) ($n=4$)
Unstimulated	–	0.22 ± 0.10
Histamine (1 μM) alone	39.6 ± 6.4**	0.30 ± 0.12
Rolipram (1 μM) alone	2.0 ± 0.2	0.38 ± 0.15
Rolipram (10 μM) alone	2.7 ± 1.5	0.42 ± 0.16
Rolipram (1 μM)+Histamine (1 μM)	50.2 ± 6.9	0.62 ± 0.24
Rolipram (10 μM)+Histamine (1 μM)	64.2 ± 5.1*	0.77 ± 0.25
Dibutyryl cyclic AMP (10 μM)	17.0 ± 4.8	–
Dibutyryl cyclic AMP (100 μM)	32.5 ± 5.5	–
Dibutyryl cyclic AMP (1000 μM)	87.5 ± 6.1	–

[a]Induced by 10^{-8} M C5a in the presence of 5 μg ml^{-1} CB; uninhibited EPO release was in the range 15–38% of total cell content. **$P<0.01$ (compared with uninhibited release); *$P<0.05$ (compared with the sum of the values for histamine (1 μM) alone and rolipram (10 μM) alone).

0.42 ± 0.16 pmoles 10^6 cells^{-1} for rolipram (10 μM) alone and 0.77 ± 0.25 pmoles 10^6 cells^{-1} for the combination, $n=4$. Dibutyryl cyclic AMP also caused a concentration-dependent inhibition of EPO release with an IC_{50} of ~ 300 μM. At the highest concentration tested (1000 μM), the inhibition was $87.5 \pm 6.1\%$.

Discussion

Both eosinophil and mast cell degranulation are key events in the pathophysiology of allergic disease, especially bronchial asthma, and although the presence of histamine H_1 and H_2 receptors on eosinophils is well-established (Clark *et al*., 1977; Anwar & Kay 1980; Plaut & Lichtenstein 1982), the effect of histamine on human eosinophil degranulation has never been determined. In the present study, we have shown that histamine, but not L-histidine, is an effective inhibitor of C5a-induced degranulation of highly purified human blood eosinophils. Although this report is based on the use of the complement fragment C5a as the stimulus, we observed the same effects when FMLP was employed as the degranulation stimulus. This rules out the possibility that histamine could be acting by interfering with C5a binding to its receptors.

We then determined that the effect of histamine was clearly mediated by the H_2 receptors. This conclusion was based on the following findings. Firstly, the inhibition of degranulation was induced by the selective H_2 agonists – dimaprit and amthamine, but not the selective H_1 agonist – HTMT, nor the selective H_3 agonists – imetit and R(−)-α-methylhistamine. Secondly, the inhibitory effects produced by both histamine and dimaprit were significantly reversed by moderate concentrations of the selective H_2 antagonists cimetidine, ranitidine and toitidine, but not the antagonists at the H_1 and H_3 receptors (mepyramine and thioperamide, respectively). Thirdly, the rank order of potency of the agonists obtained (amthamine > histamine > dimaprit) is in agreement with what has been previously reported for this receptor subtype (Eriks *et al*., 1992).

The H_2 receptor-mediated inhibition of human eosinophil degranulation, as the present results show, is in agreement with early reports that eosinophil H_2 receptor activation resulted in the inhibition of human eosinophil chemotaxis (Clark *et al*., 1977; Wadee *et al*., 1980; Sedgwick & Busse 1990). They are also consistent with several reports of H_2 receptor-mediated inhibition of degranulation of human basophils (Lichtenstein & Gillespie 1975; Tedeschi *et al*., 1991) and neutrophils (Busse & Sosman 1976).

Further evidence characterizing the receptor mediating inhibition of eosinophil degranulation as H_2 was provided by the drug-receptor interaction analysis using the selective antagonist – cimetidine. This analysis produced a typical rightwards shift of the log concentration-effect curve of dimaprit, producing a pA_2 of 5.9 and a Schild's slope (0.98) that is not different from unity. These data are consistent with simple competitive antagonism (Arunlakshana & Schild 1959) and agree with values obtained for H_2 receptors on other tissues (Lichtenstein & Gillespie 1975; Gajtkowski *et al*., 1983; Foreman *et al*., 1985).

Histamine H_2 receptors in various tissues, including pro-inflammatory cells, are known to be linked to the adenylate cyclase, *via* G-proteins, and on activation cause cyclic AMP formation that in turn mediates their response (Plaut & Lichtenstein 1982; Leurs *et al*., 1995). In this study we have also provided evidence that human eosinophil H_2 receptors are positively coupled to the adenylate cyclase system. Both histamine and dimaprit caused a concentration-dependent increase in the intracellular cyclic AMP levels, which was completely blocked by the H_2 antagonist cimetidine, but not by a combination of H_1 and H_3 antagonists – mepyramine and thioperamide. Furthermore, the inhibition of degranulation by histamine was synergistically potentiated by the PDE IV inhibitor – rolipram. Inhibition of the enzyme PDE IV, which is the predominant PDE isoenzyme in eosinophils (Barnes 1995), results in the elevation of intracellular cyclic AMP – a known inhibitor of eosinophil degranulation (Kita *et al*., 1991). Thus, expectedly, the interaction of histamine with rolipram on the inhibition of degranulation was also reflected in their combined cyclic AMP response, although in the latter the effect was additive rather than synergistic. Further support for a cyclic AMP-mediated effect was provided by the fact that dibutyryl cyclic AMP – a cell-permeable analogue of cyclic AMP – was a very effective inhibitor of degranulation. Taken together, these results suggest that histamine produced its inhibitory effect *via* H_2 receptor-mediated elevation of intracellular cyclic AMP.

The *in vivo* relevance of H_2 receptor-mediated inhibition of eosinophil function is presently uncertain but of potential significance. Histamine is the major mediator of mast cells and in an allergic reaction such as occurs in the asthmatic lung, both histamine release and eosinophil degranulation are bound to occur together. Since the concentration at which histamine inhibited eosinophil degranulation 0.1 – 50 μM is achievable in tissues following allergic mast cell degranulation, (Adams & Lichtenstein 1979), it is reasonable to expect that histamine might play an important modulatory role by down-regulating eosinophil degranulation *via* the H_2 receptors. Indeed, exogenous administration of histamine to man has been reported to cause, *via* H_2 receptors, a significant suppression of neutrophil chemotaxis *ex-vivo* (Bury *et al*., 1992), as well as eosinophil chemotaxis and mast cell degranulation *in vivo* (Ting *et al*., 1983). There is also the implication that the administration of H_2 receptor antagonists might exacerbate allergic reactions, as has indeed been previously reported (Avella *et al*., 1978; Drazen *et al*., 1978). The clinical importance of these modulatory scenarios is yet to be properly assessed.

In summary, the current results confirm the presence of H_2 receptors on human eosinophils and show that this receptor subtype mediates the inhibitory effect of histamine on eosinophil degranulation. The molecular mechanism probably involves H_2 receptor-mediated activation of membrane adenylate cyclase and the consequent increase in intracellular cyclic AMP. *In vivo*, it is possible that this histamine-eosinophil interaction is a strategy for dampening allergic inflammation.

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