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Human Polymorphonuclear Neutrophils

Pharmacological Characterization of Histamine Receptors Mediating the Elevation of Cyclic AMP

CHRISTIAN GESPACH AND JEAN-PIERRE ABITA[1]

Institut de la Santé et de la Recherche Médicale, U. 204 Hôpital Saint-Louis, 75475 Paris Cedex 10, France

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SUMMARY

The pharmacological properties of histamine receptors mediating the stimulation of cyclic AMP in human neutrophils have been investigated. The action of histamine agonists and antagonists was studied in the presence of 0.5 mM 3-isobutyl-1-methylxanthine at 37°. Histamine ($EC_{50} = 10^{-6}$ M) elicited a 10-fold stimulation of basal cyclic AMP. The order of relative potencies of the full H_1 agonists 2-(2-pyridyl)ethylamine (PEA) and 2-(2-aminoethyl)thiazole (AET) or H_2 agonists impromidine and 4-methylhistamine (4-MH) was impromidine > histamine > 4-MH > AET > PEA. The increase in cyclic AMP caused by maximally effective concentrations of the two agonists in combination was not additive and was equal to the increase caused by histamine alone. The H_1 antagonist diphenhydramine (DPH) as well as the H_2 antagonist cimetidine caused a time-dependent inhibition of the action of histamine, giving a dose-related parallel displacement of the dose-response curve to the right, without depressing the maximal response, suggesting competitive inhibition. Schild plots were linear, with pA_2 values of 4.2 ($K_i = 65 \times 10^{-6}$ M) and 6.41 ($K_i = 0.4 \times 10^{-6}$ M) for DPH and cimetidine, respectively. At concentrations giving the half-maximal inhibition, the effects of DPH and cimetidine against histamine were additive. Another H_1 antagonist, cyproheptadine, was 30 times more potent than DPH ($K_i = 2 \times 10^{-6}$ M), and only 5 times less potent than cimetidine, whereas somatostatin (10^{-6} M) did not affect the potency and the efficacy of histamine in human neutrophils. The results indicate that all agonists and antagonists studied interact with a common histamine receptor-cyclic AMP system, specifically activated or inhibited by agents possessing H_2 receptor characteristics. This study suggests that these typical H_2 receptors, evidenced in peripheral neutrophils, can be blocked *in vivo* during H_1 and H_2 antihistamine therapy in patients with inflammatory or gastric disorders.

INTRODUCTION

Several observations suggest that cyclic AMP (1) or agents which promote endogenous (2) or exogenous (3) accumulation of cyclic AMP inhibit a variety of immunological and inflammatory events mediated by human polymorphonuclear neutrophils. Among these agents, histamine has been shown to increase cyclic AMP production, which leads to the inhibition of (a) release of lysosomal enzyme (4), (b) chemotaxis (5), and (c) neutrophilic adherence (6). In the hyperimmunoglobulin E syndrome, the neutrophilic chemotactic defect is mediated by histamine *in vivo* (7) and can be reversed by antihistamines *in vivo* (7) and *in vitro* (8). However, the precise mechanism by which cyclic AMP is produced following the interaction of histamine with neutrophils is still unknown. Two distinct types of histamine receptors have been identified and classified pharmacologically as H_1 and H_2 by means of specific antagonists, which selectively block physiological or biochemical actions of histamine on various target tissues (9). In addition, the differentiation of the two classes of receptors for histamine is also reflected by the recent development of specific agonists for H_1 and H_2 receptors (10).

The purpose of this study was to characterize the receptor specificity for the cyclic AMP production system activated by histamine in human polymorphonuclear neutrophils. We therefore examined the cyclic AMP response to histamine; AET[2] and PEA as H_1 receptor agonists, impromidine and 4-MH as H_2 receptor agonists, DPH and cyproheptadine as two H_1 receptor antagonists, and cimetidine as an H_2 receptor antagonist. The further

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[2] The abbreviations used are: AET, 2-(2-aminoethyl)thiazole; PEA, 2-(2-pyridyl)ethylamine; 4-MH, (4-methyl)histamine; DPH, diphenhydramine; IBMX, 3-isobutyl-1-methylxanthine.

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characterization of the action of histamine with neutrophils may be facilitated by the substances which influence the activation of H_1 or H_2 systems in other tissues. For example, somatostatin, which is present in human plasma (11), deactivates numerous biological processes, including cyclic nucleotide synthesis (12), and inhibits the histamine H_2 receptor-cyclic AMP system implicated in gastric acid secretion (13). The effect of somatostatin on the histamine-induced cyclic AMP formation in human neutrophils has also been considered.

MATERIALS AND METHODS

Cell preparation. Venous blood of healthy adult donors was collected with heparin (10 units/ml) and used immediately for the isolation of polymorphonuclear neutrophils by the Ficoll-Hypaque method (14). For cell preparations, siliconized glassware was used. Usually, 1.8×10^6 neutrophils per milliliter of blood were obtained ($n = 88$). Residual erythrocytes were removed by osmotic shock. Cell suspensions contained approximately 98% neutrophils with a small percentage of "contaminating" erythrocytes or platelets. After centrifugation ($500 \times g$ for 5 min), the cells were suspended in Krebs-Ringer phosphate buffer (pH 7.5). To compare the data on neutrophils and the data on gastric glands, which were isolated by an EDTA technique (13), the neutrophils were first exposed for 15 min to cold medium containing 0.24 M NaCl and 2.5 mM EDTA (pH 7.5). Thereafter, the cells were washed three times with Krebs-Ringer phosphate buffer ($500 \times g$ for 5 min). The data on EDTA-treated cells were compared with those on the standard cell preparation. Cell viability (trypan blue exclusion test) was usually found to be greater than 95%, and incubation of the cells (37° for 10 or 30 min) in the absence or presence of 10^{-4} M histamine, 10^{-2} M cimetidine, or 10^{-4} M cyproheptadine did not affect cell viability.

Assay of cyclic AMP synthesis. Cells were incubated in 0.5 ml of Krebs-Ringer phosphate (pH 7.5) containing 1% bovine serum albumin and 0.5 mM IBMX as a phosphodiesterase inhibitor. In a standard assay, cell suspensions (1 to 2×10^6 cells/ml) were incubated (37° for 10 min) with continuous gentle agitation. Since the binding of histamine to plasma proteins has been demonstrated, we verified that the cyclic AMP response to 10^{-7} to 10^{-3} M histamine was not modified by incubating the neutrophils in the absence or in the presence of 0.1, 0.5, or 2% bovine serum albumin (data not shown). After incubation of the other reaction constituents for 10 min, the reaction was initiated by the addition of 100 μl of reagents. The incubation was stopped at the time indicated by the addition of 50 μl of 11 N $HClO_4$. The precipitate was removed by centrifugation ($4000 \times g$ for 10 min) and the perchlorate ions were eliminated from the supernatant by the addition of 9 N KOH. The supernatant was then added to 6.15 mg of succinic anhydride, agitated until complete dissolution, and made up to 1 ml with 0.05 M sodium acetate buffer (pH 6.2). The cyclic AMP content of the aliquots was determined by radioimmunoassay by the use of [^{125}I]tyrosylsuccinyl-cyclic AMP and the antibody 301-8, as previously described (15). The assay was sensitive to 5 fmoles of cyclic AMP. The following compounds did not interfere with the assay for cyclic AMP: ADP, AMP, ATP, adenosine, guanosine, GMP, GDP, GTP, and cyclic GMP (15). Standard cyclic AMP and unknown samples were diluted in the incubation buffer and subjected to the usual procedure of extraction and succinylation in order to minimize any effect on the assay. This procedure takes into account any inadvertent loss of cyclic AMP during the extraction. None of the agents tested in the present study interfered with the radioimmunoassay of cyclic AMP. To measure the intracellular and extracellular cyclic AMP, cells were incubated (37° for 10 min) in the absence or in the presence of 10^{-4} M histamine and centrifuged ($2000 \times g$ for 10 sec). The cell pellet was treated first with 50 μl of 11 N $HClO_4$; the volume was then made up to 550 μl with the incubation buffer and the suspension was analyzed for cyclic AMP content. The concentration of cyclic AMP in the supernatant was measured. For the same batch of cells, the total cyclic AMP formed was also determined.

Processing of the data and statistical analyses. Data are given as picomoles of cyclic AMP produced per 10^6 neutrophils. Each value is expressed as the mean ± standard error of the mean. Regression lines were fitted to the linear portions of concentration-response curves from individual experiments, and the apparent EC_{50} and IC_{50} values, i.e., the dose required to produce, respectively, 50% of the maximal stimulation or inhibition produced by the test reagents, were calculated by the method of least squares. Antagonism was analyzed by Schild plots (16) in which antagonism is expressed by the dose ratios (DR) of agonist needed to produce half-maximal responses in the absence and presence of different concentrations of antagonists:

$$\log(DR - 1) = n \log(\text{antagonist}) - \log K_B$$

For a simple competitive antagonism, the Schild plot yields a straight line with a slope of unity. The intercept with the abscissa ($DR = 2$) is the pA_2 value ($-\log K_B$), i.e., the negative log of the receptor-antagonist apparent dissociation constant. Student's t-test was used to test the significance.

Chemicals and drugs. Histamine, DPH, cyclic AMP, and IBMX were obtained from Sigma Chemical Company (St. Louis, Mo.) and bovine serum albumin (Fraction V) from Miles Laboratories (Elkhart, Ind.). Synthetic ovine somatostatin (Lot B00926) was obtained from Beckman (Geneva, Switzerland). Impromidine, 4-MH, AET, PEA, and cimetidine were generous gifts from Drs. Durant and Ganellin of Smith Kline & French Laboratories, Ltd. (Welwyn Garden City, Hertfordshire, England), and cyproheptadine-HCl was generously donated by Dr. Le Douarec (Laboratoire Merck Sharp & Dohme-Chibret, Riom 63200, France). All other chemicals were of analytical grade.

RESULTS

Effect of time, the phosphodiesterase inhibitor IBMX, and cell concentration on cyclic AMP production stimulated by histamine. The basal cyclic AMP production in neutrophils incubated at 37° averaged 0.26 ± 0.02 pmole of cyclic AMP per 10^6 cells ($n = 16$). This basal activity compares well with the values obtained for human neutrophils by other authors (17–21). Histamine

(10^{-4} M) produced a 2.5-fold stimulation of cyclic AMP synthesis (0.65 ± 0.09 pmole of cyclic AMP per 10^6 cells) after 1 min ($p < 0.001$; $n = 9$). This was followed by a gradual decrease in cyclic AMP levels which returned to basal values within 10 min (Fig. 1). In the presence of IBMX as the phosphodiesterase inhibitor, cyclic AMP levels increased rapidly during the 1st min of incubation with 10^{-4} M histamine, attained a plateau within 1–5 min, and remained constant for up to 15 min. Maximal stimulation by 10^{-4} M histamine was obtained with 0.5 mM IBMX, since further addition of the drug did not result in any further stimulation of cyclic AMP production over basal levels. Indeed, the basal cyclic AMP levels were significantly elevated from 1.45 ± 0.1 to 2.87 ± 0.26 pmoles/10^6 cells ($n = 6$) by increasing the IBMX concentration from 0.5 to 1 mM. Consequently, 10^{-4} M histamine produced, respectively, 7.5- and 5.2-fold increases in cyclic AMP production under the two experimental conditions (Fig. 1). The maximal difference between basal and histamine-stimulated cyclic AMP production was seen with 0.5 mM IBMX; this concentration of phosphodiesterase inhibitor was thus used throughout the study. The present data on the stimulatory effect of methylxanthine on baseline cyclic AMP levels in human neutrophils are similar to previous observations (18, 20). It was verified that, in control cells and in cells incubated with histamine, the cyclic AMP generated was a linear function of neutrophil concentration from 0.12 to 7.2 million cells/ml (data not shown). The data in Table 1 show that, during the entire incubation period, the cyclic AMP synthetized was contained within the cells under basal conditions or in the presence of 10^{-4} M histamine.

Effect of various concentrations of histamine and H_1 or H_2 receptor agonists on cyclic AMP production. In the presence of 0.5 mM IBMX, histamine increased the production of cyclic AMP in human neutrophils in a dose-dependent manner. This stimulation was observed at a concentration of histamine as low as 5×10^{-8} M, and increased gradually with increasing concentration, approaching a plateau at 10^{-5} M histamine. At this concentration, histamine produced a 10-fold stimulation of cyclic AMP formation over the basal value (9.43 ± 0.60 pmoles versus 0.95 ± 0.05 pmole of cyclic AMP per 10^6 cells; $n = 45$). The potency of histamine (i.e., the concentration required for half-maximal response: EC_{50}) was about 10^{-6} M histamine.

TABLE 1

Comparison of cyclic AMP generated in the entire cell suspension and in the intracellular or extracellular compartments under basal and stimulated conditions

Neutrophils were incubated as indicated under Materials and Methods. The values given are the means ± standard error of the mean from two separate experiments performed in triplicate.

	Cyclic AMP	
	Basal	10^{-4} M Histamine
	pmoles/10^6 cells	
Cell suspension	0.98 ± 0.13	10.3 ± 0.58
Cell pellet	0.88 ± 0.08	10.6 ± 0.50
Supernatant	0.10 ± 0.01	0.36 ± 0.02

The two H_1 receptor agonists AET and PEA and the two H_2 receptor agonists impromidine and 4-MH increased cyclic AMP production in human neutrophils (Fig. 2). The dose-response curves were parallel to that of histamine, and the maximally effective concentration of each agonist caused a similar 10-fold increase in cellular cyclic AMP production. Table 2 shows that a combination of maximally effective concentrations of these agonists did not produce greater stimulation. If we define the relative potency of each agonist as a ratio (EC_{50} for histamine/EC_{50} for the agonist) and if we assign a value of 100 for histamine stimulation, the relative potencies of the analogues were as follows: impromidine (1500) > histamine (100) > 4-MH (30) > AET (1.4) > PEA (0.26).

Time course for histamine stimulation of cyclic AMP and the effects of H_1 and H_2 receptor antagonists. As shown in Fig. 3, the H_1 antagonist DPH at 10^{-3} M and the H_2 antagonist cimetidine at 10^{-4} M were able to inhibit the stimulation of cyclic AMP induced by 10^{-4} M histamine. The inhibition was observed during the 15-min incubation, with no significant lag phase. The addi-

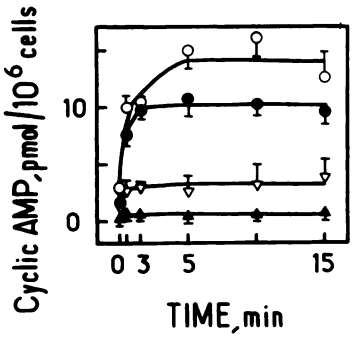

FIG. 1. *Effect of the phosphodiesterase inhibitor IBMX on the time course of cyclic AMP production induced by 10^{-4} M histamine in human polymorphonuclear neutrophils*

Cells were suspended in standard incubation solution and incubated at 37° in the absence (▲) or in the presence of 0.1 mM IBMX (▽), 0.5 mM IBMX (●), and 1 mM IBMX (○). Data are means ± standard error of the mean from a single preparation of cells; one other preparation showed similar results. Each assay was performed in triplicate.

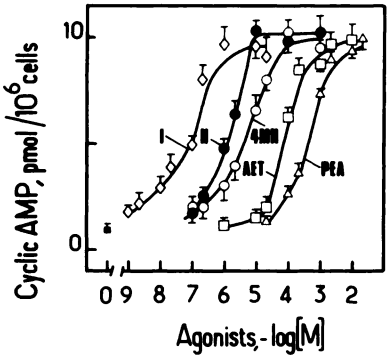

FIG. 2. *Dose-response curves for histamine and H_1 or H_2 agonists on cyclic AMP production in human polymorphonuclear neutrophils*

Cells were incubated at 37° for 10 min with the indicated concentrations of histamine (*H*, ●), impromidine (*I*, ◇), 4-MH (○), AET (□), or PEA (△) plus 0.5 mM IBMX. ■, Cyclic AMP production in control neutrophils. Data are means ± standard error of the mean from six preparations of cells. Each assay was performed in duplicate or triplicate.

TABLE 2

Effect of combination of histamine and H_1 or H_2 agonists, at maximally active concentrations, on cyclic AMP in human neutrophils

The indicated agonists were incubated at 37° for 10 min at the following concentrations: 10^{-4} M histamine, 10^{-5} M impromidine, 10^{-3} M 4-MH, 10^{-2} M AET, and 10^{-2} M PEA. Values are means ± standard error of the mean of triplicates. Two other experiments gave similar results.

Agonist	Cyclic AMP
	pmoles/10^6 cells
None	1.03 ± 0.11
Histamine	10.3 ± 0.53
Impromidine	8.6 ± 0.53
Histamine + impromidine	11.2 ± 1.13
4-MH	10.5 ± 1.08
Histamine + 4-MH	10.7 ± 0.76
AET	9.86 ± 0.77
Histamine + AET	9.44 ± 1.12
PEA	10.7 ± 0.90
Histamine + PEA	10.2 ± 1.36
PEA + impromidine	10.4 ± 0.81

tion of antagonists did not alter the time course of the increase in cyclic AMP production caused by 10^{-4} M histamine, since it became maximal and constant within 3–5 min in the absence or in the presence of the H_1 or H_2 receptor antagonists and reached a plateau within 15 min.

Effect of various concentrations of histamine and H_1 or H_2 receptor antagonists on cyclic AMP production. The inhibition of histamine-induced cyclic AMP formation by DPH and cimetidine was studied in experiments in which the concentrations of the drugs were varied in the presence of three different concentrations of histamine (10^{-6}, 10^{-5}, or 10^{-4} M) (Fig. 4). For each concentration of histamine, complete inhibition of cyclic AMP accumulation was obtained at about 10^{-3} M cimetidine (Fig. 4B) and was sustained at higher doses. Moreover, this H_2 antagonist at concentrations ranging from 10^{-7} to 10^{-2} M had no effect on the basal cyclic AMP levels.

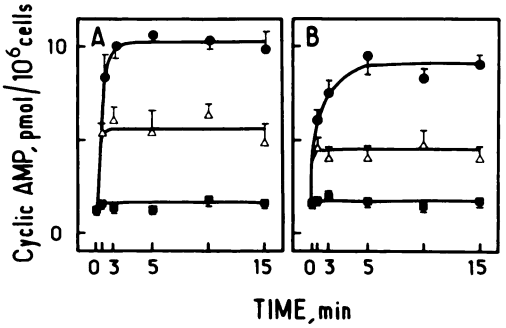

FIG. 3. *Time course for the inhibition by H_1 or H_2 antagonists of cyclic AMP production induced by 10^{-4} M histamine (●) in human polymorphonuclear neutrophils*

The inhibition was observed at 37° with 10^{-3} M DPH (A) or 10^{-4} M cimetidine (B) in the presence of 0.5 mM IBMX. Histamine and antagonists (△) were added simultaneously. ■, Cyclic AMP production in control neutrophils. Data are means ± standard error of the mean of triplicates from a single preparation of cells; one other experiment gave similar results.

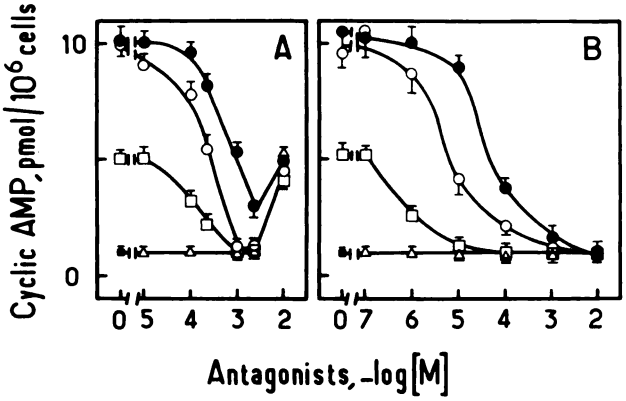

FIG. 4. *Dose-response curves for the inhibition by H_1 or H_2 antagonists of cyclic AMP production induced by histamine*

Histamine and DPH (A) or cimetidine (B) were added to the cells simultaneously and the incubation was continued for 10 min at 37°. △, Effect of increasing concentrations of DPH (A) or cimetidine (B) on basal (■) cyclic AMP production in neutrophils. Data are means ± standard error of the mean from three separate experiments performed in duplicate or triplicate. The inhibition constant (K_i) was calculated according to the equation (22) $K_i = IC_{50}/(1 + S/EC_{50})$, where IC_{50} is the concentration of antagonist required to give 50% inhibition of the histamine-stimulated increase in cyclic AMP, S is the concentration of histamine, and EC_{50} is the concentration of histamine required to give half-maximal stimulation of cyclic AMP.

A. The K_i values were obtained for DPH in the presence of histamine at 10^{-6} M (□, $K_i = 5.8 \times 10^{-5}$ M), 10^{-5} M (○, $K_i = 2.8 \times 10^{-5}$ M), and 10^{-4} M (●, $K_i = 1.6 \times 10^{-5}$ M).

B. The K_i values for cimetidine were 4.4×10^{-7} M against 10^{-6} M histamine (□), 4.7×10^{-7} M against 10^{-5} M histamine (○), and $5.4. \times 10^{-7}$ M in the presence of 10^{-4} M (●) histamine.

Strikingly, DPH at concentrations ranging from 2×10^{-3} M to 10^{-2} M increased basal cyclic AMP levels and reversed the inhibition caused by lower concentrations of this H_1 antagonist (Fig. 4). Similar results were found by Clark *et al.* (19) using another H_1 receptor antagonist, pyrilamine (10^{-4} M), upon the histamine-induced cyclic AMP generation in human eosinophils. It is also known that H_1 receptor antagonists may suppress, in a noncompetitive fashion, other actions of histamine mediated by H_2 receptors (23), and inhibit the activation of adenylate cyclase caused by sodium fluoride or by prostaglandins (24). These findings might be explained by disturbances of the integrity of the cell membrane, causing some perturbations of adenylate cyclase activity. Indeed, we have observed that incubation of neutrophils (37° for 10 min) with 10^{-2} M DPH causes a loss of cell viability, as measured by the trypan blue exclusion test. Consequently, the effect of DPH on the stimulation of cyclic AMP by histamine was studied at concentrations between 10^{-5} and 2×10^{-3} M DPH, which produced only inhibitory action and no effect on basal cyclic AMP levels.

Cyclic AMP formation stimulated by 10^{-4} M histamine was more sensitive to inhibition by the H_2 antagonist cimetidine than by the H_1 antagonist DPH ($IC_{50} = 7 \times 10^{-5}$ and 1.2×10^{-3} M, respectively). With a 10^{-6} M concentration of histamine, lower IC_{50} values were obtained for cimetidine (10^{-6} M) and DPH (10^{-4} M), suggesting that these agents were functioning as competitive

antagonists in the system. The mean K_i values (3.4×10^{-5} M DPH and 4.8×10^{-7} M cimetidine), calculated from the values obtained in Fig. 4, are indicative of a major blocking effect of cimetidine at this histamine receptor. Figure 5 also demonstrates that the H_1 and H_2 antagonists DPH and cimetidine acted as competitive inhibitors of the stimulation of cyclic AMP by histamine in neutrophils. The dose-response curves for histamine exhibited a parallel shift to the right following treatment with four different concentrations of the H_1 antagonist DPH (Fig. 5A), and at three different concentrations of the H_2 antagonist cimetidine (Fig. 5B), without change in the maximal response to histamine. The mean K_i values (5.7×10^{-5} M DPH and 3.5×10^{-7} M cimetidine) were calculated from the values obtained in Fig. 5. The data from four experiments were plotted in Fig. 6 as log ($DR-1$) against $-$(log concentration of the antagonist). The dose ratio was obtained from four-point dose-response curves. The Schild plots (16) were linear for DPH and cimetidine antagonisms ($p < 0.001$). The regression coefficients were $r = 0.982$ and $r = 0.975$, respectively. The slopes of the regression lines were 1.25 for DPH and 1.02 for cimetidine and did not differ significantly from unity, indicating a simple competition mechanism between histamine and the H_1 and H_2 antagonists within the range of concentrations tested. The affinity of the antagonists, expressed as pA_2 values (the point of intersection of the regression line and abscissa), were estimated to be 4.2 for DPH ($K_i = 6.5 \times 10^{-5}$ M) and 6.41 for cimetidine ($K_i = 4 \times 10^{-7}$ M). The inhibition constants obtained for DPH and cimetidine are in agreement with the mean K_i values determined from the experimental design of Figs. 4 and 5. As shown in Table 3, the inhibition caused by the H_1 antagonist DPH and by the H_2 antagonist cimetidine were additive at concentrations which produced the half-maximal inhibition of cyclic AMP production induced by 10^{-4} M histamine.

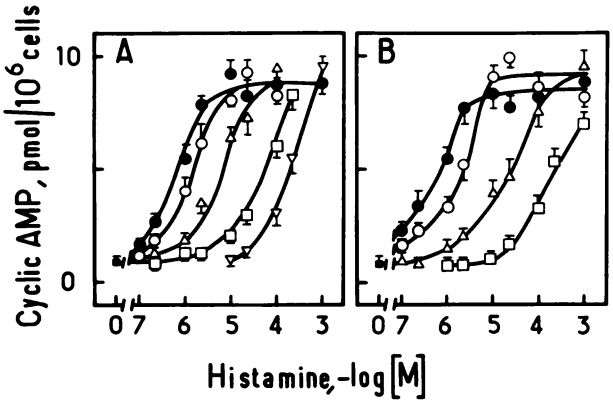

FIG. 5. *Inhibition by H_1 or H_2 antagonists of cyclic AMP production induced by various concentrations of histamine in human polymorphonuclear neutrophils*

Cells were incubated for 10 min at 37° in the presence of histamine alone (●) or in combination with DPH (A) of cimetidine (B) at the concentrations indicated below. Data are means ± standard error of the mean of triplicates from a single experiment. Comparable cyclic AMP changes were observed with cells of three other donors. The inhibition constant (K_i) was calculated (22) according to the equation $K_i = I/(k'a/ka)-1$ where $k'a$ and ka are the concentrations of histamine required to give half-maximal stimulation of cyclic AMP production in the presence and absence of antagonist, respectively, and I is the concentration of antagonist.

A. The K_i values were obtained for DPH in the presence of 10^{-4} M (○, $K_i = 9 \times 10^{-5}$ M), 4×10^{-4} M (△, $K_i = 5 \times 10^{-5}$ M), 10^{-3} M (□, $K_i = 2 \times 10^{-5}$ M), and 2×10^{-3} M DPH (▽, $K_i = 7 \times 10^{-5}$ M).

B. The K_i values were obtained for cimetidine in the presence of 10^{-6} M (○, $K_i = 3.9 \times 10^{-7}$ M), 10^{-5} M (△, 3.2×10^{-7} M), and 10^{-4} M cimetidine (□, $K_i = 3.6 \times 10^{-7}$ M).

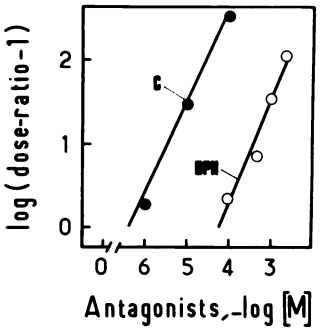

FIG. 6. *pA_2 values for cimetidine (C, ●) and DPH (○) antagonism of histamine-stimulated cyclic AMP production in human polymorphonuclear neutrophils*

Cyclic AMP values were obtained with four separate concentrations of histamine in the absence and in the presence of cimetidine or DPH at the concentrations indicated. Dose ratios were estimated graphically from the parallel displacement of straight portions of four concentration-response curves similar to data in Fig. 5. Slopes for least-squares fit were 1.02 for cimetidine (95% confidence limits = 0.92 to 1.12) and 1.25 for DPH (95% confidence limits = 1.1–1.4).

The inhibition of histamine-induced elevation of cyclic AMP in peripheral blood neutrophils by another H_1 receptor antagonist, cyproheptadine, was studied in preliminary experiments of this type. It was found that 10^{-6}–10^{-4} M cyproheptadine also completely blocked the stimulation produced by 10^{-5} or 10^{-6} M histamine. However, at these concentrations cyproheptadine had no effect on basal cyclic AMP levels in neutrophils, and the analysis of concentration inhibition curves gave a K_i of about 2×10^{-6} M cyproheptadine (data not shown).

Effect of somatostatin on the dose-response curve for histamine-induced cyclic AMP generation in human

TABLE 3

Effect of combination of histamine with H_1 or H_2 antagonists at concentrations which produce half-maximal inhibition of cyclic AMP production in the presence of 10^{-4} M histamine

The indicated antagonists were incubated in standard conditions at the following concentrations: In the presence of 2×10^{-5} M cimetidine, the addition of 10^{-3} M DPH produced additional inhibition of cyclic AMP formation ($p < 0.001$ versus respective controls). Histamine and antagonists were added simultaneously. Values are means ± standard error of the mean of triplicates. Two other experiments gave similar results.

Additive	Cyclic AMP
	pmoles/10⁶ cells
None	0.97 ± 0.13
Histamine	8.13 ± 0.20
Histamine + cimetidine	4.86 ± 0.13
Histamine + DPH	3.30 ± 0.08
Histamine + cimetidine + DPH	1.77 ± 0.08

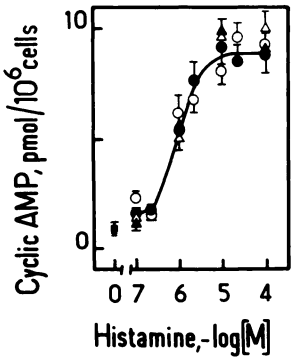

FIG. 7. *Effect of somatostatin and EDTA on cyclic AMP stimulation by histamine in human neutrophils*

Histamine alone (*closed symbols*) or histamine plus 10^{-6} M somatostatin (*open symbols*) were added to EDTA-treated (▲, △) and control cells (●, ○). Date are means ± standard error of the mean from two preparations of cells.

neutrophils. Some experiments were carried out on human neutrophils to study the effect of 10^{-6} M somatostatin on the histamine dose-response curves. The data in Fig. 7 show that somatostatin did not affect either the potency or the efficacy of histamine on the cyclic AMP response in control and EDTA-treated neutrophils (see Materials and Methods).

DISCUSSION

The data reported here demonstrate that in the presence of IBMX, a potent phosphodiesterase inhibitor, histamine causes a rapid and significant stimulation of cyclic AMP production in human polymorphonuclear neutrophils. The steady-state concentration in cyclic AMP obtained under these optimal conditions facilitates the study of the dose-response relationships for histamine agonists or antagonists in human neutrophils. Biologically relevant doses of histamine (5×10^{-8} to 10^{-5} M) caused a monophasic concentration-dependent increase in cyclic AMP (10-fold the basal value), with the EC_{50} observed at 10^{-6} M histamine. These effects of methylxanthine as well as of histamine on cyclic AMP levels in human neutrophils agree with previous observations (17–20). Hence, it was shown by Stolc (25) that histamine is a positive effector on the membrane-bound adenylate cyclase in human polymorphonuclear leukocytes. In contrast, Clark *et al.* (19) and Marone *et al.* (21) found that human neutrophils are only marginally responsive to histamine, with respect to cyclic AMP generation. This apparent discrepancy can be explained by the fact that these experiments were conducted in the absence of any phosphodiesterase inhibitor, and during 10-min incubation periods, when cyclic AMP concentrations in neutrophils have returned to basal levels, as shown in this paper. Our studies were conducted with a preparation of human neutrophils purified from the subpopulations of leukocytes. Indeed, previous reports imply that histamine also increases the cyclic AMP content of eosinophils and lymphocytes (19–21), probably via an H_2 receptor. It has also been shown that human neutrophils and lymphocytes differ both in basal cyclic AMP content and in response to histamine (17, 18, 20, 21). The observation that the total cyclic AMP generated by human neutrophils in response to histamine was contained in the cell pellet suggests that cyclic AMP acts at the interior of the neutrophils through intracellular components such as protein kinase, rather than by an extracellular mechanism. As in a previous study (26), it has been demonstrated recently that the release of cyclic AMP *in vivo* by endothelial cells after epinephrine stimulation attenuates neutrophilic adherence to the endothelial vasculature (3). Thus, cyclic AMP may also inhibit neutrophilic function by a cell surface interaction *in vivo*.

The series of experiments described in this study represent the first investigation of the receptor specificity for histamine-induced elevation of cyclic AMP in human neutrophils. The pharmacological analysis of the effects of the histamine agonists and antagonists tested on cyclic AMP production in human neutrophils shows a potency sequence characteristic of the H_2 type histamine receptor. First, we found that in the system studied the four H_1 and H_2 analogues act as full agonists of histamine action. The dose-response curves were monophasic and paralleled that of histamine, indicating that these analogues interact with a set of receptors having similar efficacy and density for coupling to the histamine-sensitive adenylate cyclase (25) in human neutrophils. In agreement, the effect produced on human neutrophils by histamine or H_1 and H_2 agonists in combination were not additive. The order of potency of these agonists in human neutrophils (impromidine > histamine > 4-MH > AET > PEA) is also the order of their relative potencies ($H_2 > H_1$) in other systems in which the effects of histamine are mediated through H_2 receptors (27), whereas an inverse sequence ($H_1 > H_2$) was found for H_1 receptors (28). Accordingly, the specific histamine H_2 receptor agonist impromidine, which has 9–48 times the potency of histamine on tissues known to contain histamine H_2 receptors, failed to exert an agonistic effect at the level of H_1 receptors in guinea pig ileum (29). Second, the action of histamine on cyclic AMP production in human neutrophils could be inhibited by H_1 as well as by H_2 receptor antagonists. The antagonism was found to be competitive. The pA_2 values of 4.2 ($K_i = 65 \times 10^{-6}$ M) and 6.41 ($K_i = 0.4 \times 10^{-6}$ M) were calculated for DPH and cimetidine, respectively. This represents a blocking potency for cimetidine of approximately 100 times that of DPH ($H_2 > H_1$). These values suggest a greater affinity of cimetidine for the histamine receptors in neutrophils than has been reported for the H_2 receptor-mediated stimulation of cyclic AMP formation in guinea pig ventricular muscle, $K_i = 10^{-6}$ M (27). In contrast, comparable K_i values ($K_i = 0.4 \times 10^{-6}$ M) were found on H_2 receptors in rat (13, 30) and guinea pig ($K_i = 0.3 \times 10^{-6}$ M) parietal cells (31). On the other hand, the inhibitory potency of DPH in the present work compares well with the potency with which this H_1 antagonist inhibits the action of histamine on H_2 receptors (K_i values $3.7–5 \times 10^{-5}$ M) in rat (30) and guinea pig (31) parietal cells. In contrast, DPH was approximately 1000 times more potent on typical H_1 receptors (32). The H_1 receptor antagonist cyproheptadine also inhibited the action of histamine in human neutrophils. The calculated K_i value (2×10^{-6} M cyproheptadine) agrees with that obtained against the

histamine H_2 receptors in sections of guinea pig hippocampus (33) and confirms the above observations that histamine exerts its biological effect in human neutrophils via a receptor-cyclic AMP system preferring H_2 conformations. The present results clearly show that somatostatin does not interfere with the histamine-stimulated cyclic AMP formation in human neutrophils; instead, somatostatin inhibits the H_2 receptor-cyclic AMP system in rat and guinea pig gastric glands (13, 31). The biological significance of specific receptors for somatostatin in circulating human blood cells (34) thus remains to be elucidated.

Our results are in accord with those of Busse and Sosman (4), who found that histamine (10^{-7}–10^{-5} M) increased cyclic AMP production, which paralleled the inhibition of β-glucuronidase release in human neutrophils. In contrast, the inhibitory effect of histamine on chemotaxis could be observed at concentrations from 10^{-6} to 10^{-4} M, and was half-maximal at 6.5×10^{-6} M histamine (5), a dose which corresponds to the submaximal stimulation of cyclic AMP production by histamine in the present experiments. Since the anti-inflammatory action of histamine, i.e., the inhibition of the release of lysosomal enzymes and chemotaxis (4, 5) could be abolished, at the doses investigated, by H_2 receptor antagonists but not by H_1 antihistamines, it has been suggested that the above-mentioned inhibitory effects are mediated by an H_2 receptor. In these studies, DPH at a concentration of 2.5×10^{-4} M had no effect on the inhibition of true chemotaxis upon exposure of neutrophils to 2.5×10^{-5} M histamine (5). Similarly, the data in Fig. 5A demonstrate that those relative concentrations of histamine and H_1 antagonist had only a minor effect on the maximal response elicited by histamine. However, the H_1 antagonist cyproheptadine was approximately 30 times more potent than DPH and only 5 times less potent than cimetidine at this histamine receptor. Thus, one could postulate the effectiveness of the H_1 antagonists against the action of histamine in human neutrophils. This postulation is also supported by the contribution of the H_1 receptor blocker DPH when combined with cimetidine at a concentration producing half-maximal inhibition of the histaminergic stimulation. The present findings indicate that the cyclic AMP generation induced by histamine in human neutrophils involves the stimulation of typical H_2 receptors which can be blocked *in vitro* and *in vivo* by H_1 or H_2 antagonists such as cimetidine, DPH, or cyproheptadine in patients with pruritus (35) or ulcers (36). The mean concentration of cimetidine in blood is reported to be in the range of $4–8 \times 10^{-6}$ M cimetidine during the first 8 hr after the standard dose of cimetidine (1 g/day) (36). These concentrations of cimetidine measured in peripheral blood are 10 times higher than the inhibition constant of cimetidine for histamine-stimulated cyclic AMP production in human neutrophils. Hence, the K_i value for cyproheptadine inhibiting the response to histamine was 2×10^{-6} M. This concentration approximates that reported for circulating blood levels of cyproheptadine after its oral administration in man (37).

The potency and efficacy of histamine on cyclic AMP production in human neutrophils provide a particularly useful model for screening putative H_1 and H_2 receptor agonists and antagonists at the level of human tissue by the use of peripheral blood neutrophils, so readily accessible. With the present *in vitro* technique, the results were obtained by the use of 0.5×10^{-6} neutrophils per assay tube. About 20 ml of blood were required for each experiment, thus allowing repetitive blood collection from patients. Our investigation thus provides a molecular basis for obtaining information on the mechanism of desensitization of histamine receptors during H_1 and H_2 antihistamine therapy in man.

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Send reprint requests to: Dr. C. Gespach, Institut de la Santé et de la Recherche Médicale, U. 204, Hôpital Saint-Louis, 2 place du Docteur Fournier, 75475 Paris Cedex 10, France.