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Identification and Characterization of Surface Receptors for Histamine in the Human Promyelocytic Leukemia Cell Line HL-60

Comparison with Human Peripheral Neutrophils

CHRISTIAN GESPACH,[1] FORTUNA SAAL,[2] HÉLÈNE COST,[1] AND JEAN-PIERRE ABITA[1]

Institut de la Santé et de la Recherche Médicale, U.204 and U.107, Hôpital Saint-Louis, 75475 Paris Cedex 10, France

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SUMMARY

The magnitude, the potency, the duration, and the specificity of histamine-induced cyclic AMP formation has been compared in human promyelocytic leukemic HL-60 cells and in human peripheral neutrophils. In HL-60 cells incubated at 37° in the absence of phosphodiesterase inhibitor, histamine caused a 20-fold stimulation of basal cyclic AMP levels, with an EC_{50} of 5×10^{-6} M. Typical H_2 receptors were involved as shown by the relative potencies of the H_1-selective agonists, 2-(2-pyridyl)ethylamine (PEA) and 2-(2-aminoethyl)thiazole (AET), and the H_2-selective agonists, impromidine and 4-methyl)histamine(4-MH): impromidine > histamine > 4-MH > AET > PEA. In this system, impromidine had mixed agonist-antagonist properties as shown by the rightward shift of the concentration-response curve of histamine ($EC_{50} = 2 \times 10^{-3}$ M histamine in the presence of 10^{-4} M impromidine). Histamine stimulation was competitively inhibited by the furane derivative ranitidine ($K_i = 0.16 \times 10^{-6}$ M) as well as by the imidazole analogues oxmetidine ($K_i = 0.48 \times 10^{-6}$ M) and cimetidine ($K_i = 0.65 \times 10^{-6}$ M), whereas the H_1 antagonist diphenhydramine inhibited histamine action at about 100–300 times higher concentrations ($K_i = 51 \times 10^{-6}$ M). Prostaglandin E_1 (PGE_1) also stimulated cyclic AMP levels (50-fold increase) in HL-60 cells; half-maximal activation by PGE_1 occurred at 3.2×10^{-6} M. Our results indicate, first, that prostaglandin and histamine H_2 receptors are present and functional at an early stage during myeloid differentiation; second, that there is no substantial difference between the pharmacological properties of the histamine H_2 receptors in HL-60 cells and in mature human peripheral neutrophils; third, that the remarkable capacity for cyclic AMP formation noted in HL-60 leukemic cells after cell surface interaction by histamine or prostaglandin suggests that cyclic AMP and agents which increase its formation may have a role in the regulation of proliferation and/or differentiation of human myeloid progenitor cells.

INTRODUCTION

Histamine, acting via H_2 receptors, has been shown to accelerate proliferation of hemopoietic stem cells *in vitro* (1, 2). Accordingly, administration of the H_2 receptor antagonist metiamide or cimetidine during treatment of peptic ulcer disease and Zollinger-Ellison syndrome was followed by reversible agranulocytosis (see ref. 2 for references), suggesting the presence of surface receptors for histamine at the level of the myeloid precursor cells in bone marrow. To investigate this possibility, we examined changes in cyclic AMP levels in human promyelocytic leukemic HL-60 cells after histamine administration. This cell line, established from the peripheral blood of a patient with acute leukemia (3), consists predominantly of promyelocytes and can be induced to undergo myeloid differentiation to granulocytes (4) having the ability to phagocytose, lysosomal enzyme release, chemotaxis, and superoxide generation (5, 6). Therefore, this HL-60 cell line is a good model with which to study cell surface receptors at the level of the granulocytic precursors in man.

In the present study, the magnitude, the potency, the duration, and the specificity of the increases in cyclic AMP levels evoked by histamine were determined in HL-60 cells incubated in the absence and presence of IBMX[3]

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[1] Institut de la Santé et de la Recherche Médicale, U.204.

[2] Institut de la Santé et de la Recherche Médicale, U.107.

[3] The abbreviations used are: IBMX, 3-isobutyl-1-methylxanthine; AET, 2-(2-aminoethyl)thiazole; PEA, 2(2-pyridyl)ethylamine; 4-MH, 4-(methyl)histamine; DPH, diphenhydramine; PGE_1, prostaglandin E_1.

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as a phosphodiesterase inhibitor. To establish the pharmacological specificity of histamine interaction on HL-60 cells in terms of H_1 and H_2 receptor dependence, we investigated the cyclic AMP responses to histamine, AET, and PEA as selective H_1 receptor agonists; impromidine and 4-MH as selective H_2 receptor agonists; DPH as an H_1 receptor antagonist; and cimetidine, oxmetidine, and ranitidine as H_2 receptor antagonists. Results were analyzed with special reference to the histamine H_2 receptor-cyclic AMP system that we previously characterized in mature peripheral granulocytes in man (7). In addition, the effect of PGE_1, a ubiquitous agonist of membrane-bound adenylate cyclase, was compared with that of histamine in HL-60 cells and in human peripheral neutrophils.

MATERIALS AND METHODS

Reagents. Histamine, diphenhydramine, prostaglandin E_1, cyclic AMP, and IBMX were obtained from Sigma Chemical Company (St. Louis, Mo.) and bovine serum albumin (Fraction V) from Miles Laboratories (Elkhart, Ind.). Fetal calf serum, RPMI-1640 medium, and antibiotics were purchased from Laboratoire Flow (Puteaux, France). Impromidine, 4-MH, AET, PEA, cimetidine, and oxmetidine were generous gifts from Dr. Brimblecombe, of Smith Kline & French Laboratories Ltd. (Welwyn Garden City, Hertfordshire, England); ranitidine was kindly donated by Dr. C. Alexandre (Laboratoires Glaxo, Paris, France).

HL-60 cells. Human promyelocytic HL-60 leukemia cells were generously provided by Dr. R. C. Gallo (National Cancer Institute, Bethesda, Md.). The cells are passaged at starting densities of $1.5–2 \times 10^5$ cells/ml and maintained in suspension culture in RPMI-1640 supplemented with 20% fetal calf serum, L-glutamine (2 mM), streptomycin (200 μg/ml), and penicillin (200 IU/ml), as previously described (3). The cell cultures were split every 3 or 4 days, so that the cell density was maintained between 0.5 and 1.5×10^6 cells/ml. The population doubling time was approximately 48 hr. The experiments reported here were performed on cells between Passage 9 and Passage 60. Cells were harvested by centrifugation ($200 \times g$ for 5 min), washed twice in Krebs-Ringer phosphate buffer (pH 7.4), and finally resuspended in the same buffer to a final concentration of $2–4 \times 10^6$ cells/ml for cyclic AMP synthesis. Cells were counted in a hemocytometer chamber, and cell viability was determined by trypan blue dye exclusion. Differential counts were performed on a minimum of 100 cells for each experiment. Cell viability was 80–95%, and incubation of HL-60 cells (37° for 10 or 30 min) in the absence or presence of 10^{-4} M histamine, 10^{-2} M cimetidine, or DPH did not affect cell viability.

Human neutrophils. Peripheral blood neutrophils from normal donors were obtained by the Ficoll-dextran method (8) and consisted of >95% mature polymorphonuclear neutrophils. Before cyclic AMP assay, cells were washed twice and resuspended in Krebs-Ringer phosphate buffer at the final concentration of $4–6 \times 10^6$ cells/ml.

Cyclic AMP assay. In a standard assay, HL-60 cells ($0.6–1.2 \times 10^6$ cells/ml) or human neutrophils ($1–2 \times 10^6$ cells/ml) were incubated at 37° with continuous gentle agitation in 0.5 ml of Krebs-Ringer phosphate buffer (pH 7.4) containing 1% bovine serum albumin. Incubations were carried out in the absence or in the presence of 0.5 mM IBMX as a competitive inhibitor of the cyclic AMP phosphodiesterases. Cyclic AMP in HL-60 cells or in human neutrophils was determined by radioimmunoassay, as previously described (7). All determinations were made in duplicate or triplicate.

Expression of results and statistical analysis. Data were normalized as the percentage of the response to a given concentration of stimulant, and absolute values are given as picomoles of cyclic AMP produced by 10^6 cells. The apparent EC_{50} and IC_{50} values were the doses of agonists or antagonists required to produce, respectively, 50% of the maximal stimulation or inhibition by the test agents.

Antagonism by different concentrations of H_1 or H_2 antihistamines against a fixed concentration of histamine was analyzed according to the following equations (9):

$$K_i = IC_{50}/(1 + S/EC_{50})$$

where K_i is the inhibition constant of antagonist, IC_{50} is the concentration of antagonist required to give 50% inhibition of the histamine-stimulated increase in cyclic AMP, S represents the concentration of histamine, and EC_{50} is the concentration of histamine required to give 50% stimulation of cyclic AMP; and

$$K_i = I(K'_a/K_a) - 1)$$

where K_a and K'_a are the concentrations of histamine required to produce half-maximal cyclic AMP stimulation in the absence and presence of antagonist, respectively, and I is the concentration of antagonist. Results were analyzed by standard methods using Student's paired t-test.

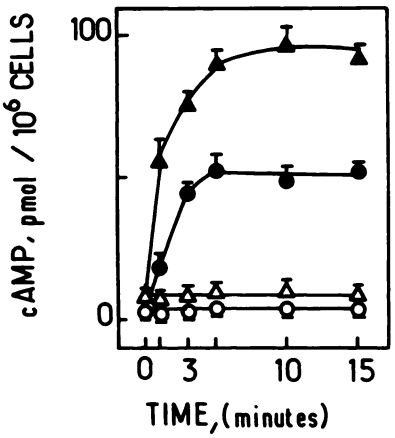

FIG. 1. *Effects of time and the phosdiesterase inhibitor IBMX on basal and histamine-stimulated cyclic AMP production in HL-60 cells*

Cells ($0.6–1.2 \times 10^6$/ml) were preincubated at 37° for 10 min in standard solution in the absence (○, ●) or in the presence (△, ▲) of 0.5 mM IBMX, and then incubated for the time indicated, without (control: ○, △) or with 10^{-4} M histamine (●, ▲). Data are means ± standard error of the mean of results from five experiments performed in duplicate.

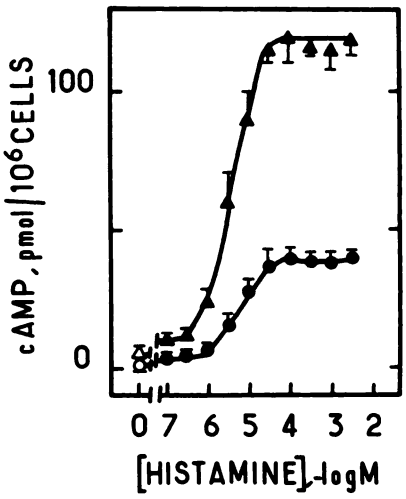

FIG. 2. *Effect of various concentrations of histamine on the level of cyclic AMP in HL-60 cells incubated in the absence or in the presence of IBMX*

Cells were incubated at 37° for 10 min in standard solution in the absence (●) or in the presence (▲) of 0.5 mM IBMX, and then incubated for 5 min with histamine. Data are means ± standard error of the mean of results from 11 experiments without IBMX and from 4 experiments with IBMX. The addition of the phosphodiesterase inhibitor did not change significantly the potency of histamine in the system.

RESULTS

Influence of time and IBMX on cyclic AMP levels stimulated by histamine in HL-60 cells. In the human promyelocytic HL-60 cell line, cellular cyclic AMP levels were 1.72 ± 0.07 pmoles/10^6 cells (n = 37) during a 15-min incubation period at 37° (Fig. 1). After the addition of 10^{-4} M histamine, the cyclic AMP concentration increased within 1 min; half-maximal and maximal stimulations were observed within 2 and 5 min, respectively. Maximal cyclic AMP stimulation by 10^{-4} M histamine represented a 20-fold increase over basal values (34.4 ± 1.5 pmoles/10^6 cells, n = 37) and cyclic AMP levels remained constant for the remainder of the 15-min incubation (Fig. 1). In cells incubated for 5 min with 0.5 mM IBMX as a competitive inhibitor of cyclic AMP-phosphodiesterase activity, basal and histamine-stimulated cyclic AMP levels were 7.68 ± 0.64 and 70.6 ± 5.8 pmoles of cyclic AMP per 10^6 cells, respectively (n = 15). Under these experimental conditions, half-maximal and maximal cyclic AMP stimulations by 10^{-4}M histamine were observed within 2 and 5 min, respectively, reaching a steady state within 5–15 min.

Dose-effect of histamine on cyclic AMP levels in HL-60 cells incubated in the absence or in the presence of IBMX. Cyclic AMP production in HL-60 cells incubated during 5 min at 37° was stimulated by histamine over a range of concentrations from 10^{-7} to 3×10^{-5} M histamine (Fig. 2). Half-maximal stimulations were observed at 5×10^{-6} or 3×10^{-6} M histamine in the absence or in the presence of IBMX, respectively. In both cases, the dose-response curves were monophasic, and increasing histamine concentrations up to 3×10^{-3} M did not change the plateau values. We have verified that under basal conditions and after the addition of 10^{-4} M histamine, cyclic AMP levels in HL-60 cells incubated with or without IBMX were linearly correlated with the number of cells in a wide range from 0.22 to 6×10^6 cells/ml (data not shown). A comparison of the basal or histamine-stimulated cyclic AMP production in HL-60 cells and in human neutrophils (7) is presented in Table 1.

Dose effect of PGE_1 on cyclic AMP levels in HL-60 cells and in human neutrophils. Basal cyclic AMP levels in HL-60 cells (1.83 ± 0.37 pmoles/10^6 cells) were significantly increased by 10^{-7} M PGE_1; half-maximal and maximal stimulations by PGE_1 (90.8 ± 5.87 pmoles/10^6 cells, i.e., a 50-fold increase over basal; n = 3) were observed at 3.2×10^{-6} M and 4×10^{-5} M PGE_1, respectively (Fig. 3). Figure 3 also shows that, in peripheral neutrophils, PGE_1 (10^{-8}–10^{-5} M) produced a 6-fold stimulation of cyclic AMP production over basal values (8.24 ± 0.34 versus 1.38 ± 0.15 pmoles of cyclic AMP per 10^6 cells; n = 6). The potency of PGE_1 was about 10^{-6} M. These results compare well with the data obtained by Galant *et al.* (10) on the PGE_1-induced cyclic AMP generation in human neutrophils.

Comparative effects of various H_1 or H_2 histamine receptor agonists on cyclic AMP levels in HL-60 cells. The effects of impromidine and 4-MH, two H_2-selective agonists, and of PEA and AET, two H_1-selective agonists, on cyclic AMP accumulation in HL-60 cells were compared with those of histamine (Fig. 4, *left*). Data are presented as percentages of the maximal stimulation elicited by the maximally effective compound. We verified that the time course of response to the H_2-selective agonist impromidine (10^{-5} M) or to the H_1 selective agonist AET (10^{-2} M) was similar to that obtained with histamine. The highly selective histamine H_2 receptor agonist impromidine (11) stimulated cyclic AMP accumulation in HL-60 cells with an EC_{50} value of 1.6×10^{-7} M, that is, approximately 44 times lower than that of histamine. Impromidine exerted its maximal stimulation at 10^{-6} M. At this H_2 agonist concentration, basal cyclic AMP levels were elevated from 1.51 ± 0.05 to 4.76 ± 0.28

TABLE 1

Comparison of basal and histamine-stimulated cyclic AMP production in HL-60 cells and in human neutrophils (7) incubated in the absence or in the presence of IBMX, as a phosphodiesterase inhibitor

Cells	Cyclic AMP			
	Without IBMX		With IBMX (0.5 mM)	
	Basal	Histamine (10^{-4} M)	Basal	Histamine (10^{-4} M)
	pmoles/10^6 cells			
HL-60 cells	1.72 ± 0.07	34.4 ± 1.5	7.68 ± 0.64	70.6 ± 5.8
Human neutrophils	0.26 ± 0.02	0.65 ± 0.09	0.95 ± 0.05	9.43 ± 0.6

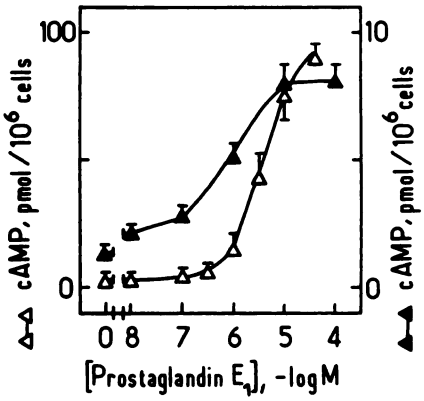

FIG. 3. *Dose-dependence of the effect of PGE_1 on cyclic AMP levels in HL-60 cells and in human neutrophils*

HL-60 cells (△) were incubated for 5 min at 37° in the absence of IBMX, as described under Materials and Methods. Human neutrophils (▲) were incubated for 10 min at 37° after a 10-min preincubation period in the incubation buffer containing 0.5 mM IBMX (7). In HL-60 cells and in human neutrophils cyclic AMP levels were maximal and constant during the 3- to 15- or 5- to 15-min incubation period after the addition of 10^{-5} M PGE_1, respectively. For HL-60 cells, three experiments were performed in duplicate or triplicate; for peripheral neutrophils, six experiments were performed in triplicate. Data are means ± standard error of the mean for each agonist concentration tested.

pmoles/10^6 cells (n = 3), and this effect represents only 15–20% of the maximal stimulation obtainable with histamine. In contrast, the H_2-selective agonist 4-MH and the H_1-selective agonists AET and PEA were able to exert the same maximal stimulation observed with histamine. For these three agonists, the dose-response curves were monophasic and paralleled that of histamine.

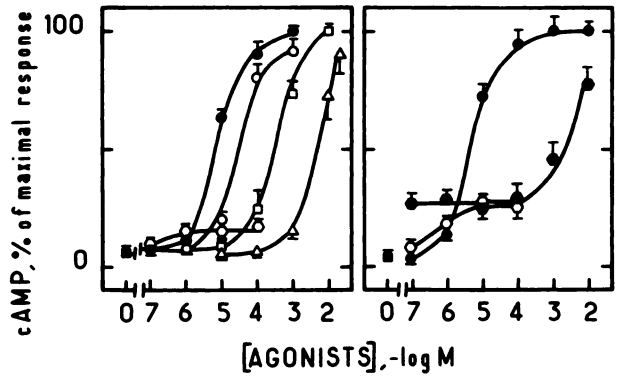

FIG. 4. *Dose-response curves for histamine and H_1 and H_2 agonists alone or in combination on the level of cyclic AMP in HL-60 cells*

Left. Cells were preincubated at 37° for 10 min in standard solution in the absence of IBMX and then incubated for an additional 5 min with the indicated concentrations of histamine (●), impromidine ( ), 4-MH (○), AET (□), or PEA (△). Basal cyclic AMP production (■) was measured in HL-60 cells.

Right. Effect of various concentrations of histamine alone (●) or in combination with 10^{-4} impromidine (⬣) on cyclic AMP formation in HL-60 cells. Basal (■) and impromidine-stimulated (○) cyclic AMP production was measured in the same preparations.

Data are expressed as the percentage of the maximal cyclic AMP production elicited by the agonists (left, 29.9 ± 1.12 pmoles of cyclic AMP per 10^6 cells; right, 35.1 ± 2.3 pmoles of cyclic AMP per 10^6 cells) and represent the means ± standard error of the mean of results from three experiments performed in duplicate or triplicate.

A comparison of the relative potencies of the histamine analogues on histamine receptors mediating cyclic AMP accumulation in HL-60 cells and in human neutrophils (7) is presented in Table 2. To examine further the action of the partial agonist impromidine with the histamine receptor in HL-60 cells, impromidine at 10^{-4} M was added simultaneously with different concentrations of histamine, from 10^{-7} M to 10^{-2} M (Fig. 4, *right*). Impromidine displaced to the right the histamine concentration curve: 50% of the maximal cyclic AMP rise was observed at histamine concentrations of 5×10^{-6} and 2×10^{-3} M in the absence and in the presence of impromidine, respectively, giving a K_i value of 0.25×10^{-6} M impromidine. In these experiments, impromidine alone produced 20% (9.21 ± 1.1 pmoles/10^6 cells; n = 3) of the maximal histamine stimulation.

Comparative effects of various H_1 and H_2 histamine receptor antagonists on cyclic AMP levels stimulated by histamine in HL-60 cells. Figure 5 shows the effects of various concentrations of the H_1 antagonist DPH or the H_2 antagonists cimetidine, oxmetidine, and ranitidine on cyclic AMP production stimulated by 10^{-5} or 10^{-4} M histamine. The cyclic AMP response to a fixed concentration of histamine (10^{-5} M) was completely inhibited in a monophasic manner by the H_2 antagonist cimetidine in increasing concentrations from 10^{-7} to 10^{-4} M (Fig. 5, *left*). Approximately 50% of the response was inhibited, with an IC_{50} of 2×10^{-6} M cimetidine, giving a K_i value of 0.67×10^{-6} M. The H_1 antagonist DPH also produced a dose-related inhibition of the histamine stimulation, but at 100 times higher concentrations ($IC_{50} = 2 \times 10^{-4}$ M DPH), giving a K_i value of 66×10^{-6} M. With histamine at 10^{-5} M, higher IC_{50} values were obtained for cimetidine (1.8×10^{-5} M) and for DPH (5.4×10^{-4} M), suggesting that these agents were functioning as competitive antagonists in the system. The relative potency of each H_1 or H_2 antagonist was established in Fig. 5 (*right*) as the ratio of IC_{50} for cimetidine/IC_{50} for the antagonist. If we assign a value of 100 for cimetidine inhibition, the relative potencies of the antagonists were: ranitidine (475) > oxmetidine (160) > cimetidine (100) > DPH (2).

The antagonism of histamine by the H_1 blocker DPH or by the H_2 blocker cimetidine on HL-60 cells, estimated from displacement of dose-response curves, was analyzed, as shown in Fig. 6. Cimetidine (*left*) produced a dose-related displacement to the right of the histamine concentration curve without depressing the maximal response, indicating that cimetidine is a competitive antag-

TABLE 2

Comparison of the relative potencies of histamine H_1- and H_2-selective agonists on cyclic AMP production in HL-60 cells and in human neutrophils

The relative potency of each selective agonist was established as the ratio (EC_{50} for histamine/EC_{50} for the agonist × 100). The potency of histamine (EC_{50}) in each cell preparation is indicated in parentheses as reference.

Cells	Agonist			
	Histamine (EC_{50})	4-MH	AET	PEA
HL-60 cells	100 (5×10^{-6} M)	23	2.3	0.17
Human neutrophils	100 (10^{-6} M)	30	1.4	0.26

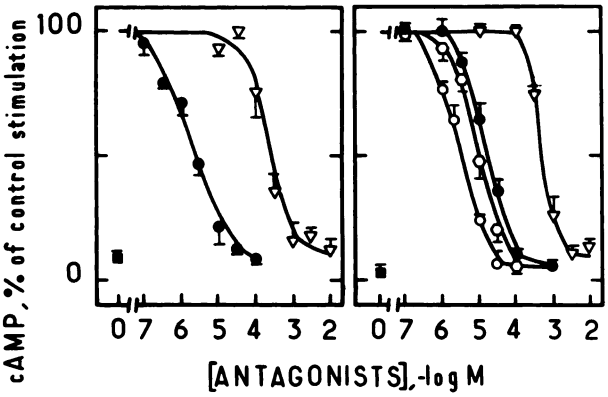

FIG. 5. *Effect of various concentrations of H_1 or H_2 antagonists on cyclic AMP production induced by 10^{-5} M (left) or 10^{-4} M (right) histamine in HL-60 cells*

After a 10-min preincubation period at 37°, histamine alone or in combination with its antagonists was added and the incubation was continued for 5 min in the absence of IBMX. Results are expressed as the percentage of cyclic AMP production elicited by histamine at 10^{-5} M (18.6 ± 2.2 pmoles of cyclic AMP per 10^6 cells) or at 10^{-4} M (32.1 ± 1.75 pmoles of cyclic AMP per 10^6 cells), in the absence of antagonist. Basal cyclic AMP levels in HL-60 cells (■) were not altered by 10^{-7}–10^{-2} M cimetidine or by 10^{-5}–10^{-3} M DPH, whereas 10^{-2} M DPH alone produced a 3-fold stimulation of basal levels, as in ref. 7.

Left. The K_i values for cimetidine (●) and DPH (▽) were 0.60 and 73×10^{-6} M, respectively. Data are means ± standard error of the mean results from three separate experiments performed in duplicate or triplicate.

Right. The K_i values for ranitidine (○), oxmetidine (◇), cimetidine (●), and DPH (▽) were 0.16, 0.48, 0.76, and 36×10^{-6} M, respectively. Data are means ± standard error of the mean of results from five to seven experiments for each H_2 antagonist and from three experiments for the H_1 antagonist DPH. Each determination was performed in duplicate or triplicate. We verified that the H_1 and H_2 antagonists tested at the concentrations giving half-maximal inhibitions did not alter the time course of the increase caused by 10^{-4} M histamine, since cyclic AMP concentrations became maximal within 3–5 min and were constant until 10 min after the addition of each drug.

onist. In the presence of 10^{-6} or 5×10^{-6} M cimetidine, the concentrations of histamine required for half-maximal stimulation of cyclic AMP increased from 6.2×10^{-6} to 1.6 and 5.6×10^{-5} M histamine, respectively. Similarly, the concentration-response curve for histamine was progressively shifted to the right in the presence of the H_1 antagonist DPH (Fig. 6, *right*) at 10^{-4} M ($EC_{50} = 1.4 \times 10^{-5}$ M histamine) or at 5×10^{-4} M ($EC_{50} = 10^{-4}$ M histamine). For each drug, the K_i was calculated from the two experimental designs of Figs. 5 and 6, assuming competitive inhibition. The results summarized in the legends to these two figures are in close agreement. The K_i values, based on the two methods of determination, were 0.65 ± 0.07 and 51 ± 10 × 10^{-6} M for cimetidine and DPH, respectively.

DISCUSSION

The data presented show that histamine and PGE_1 cause an increase in cyclic AMP levels in the human promyelocytic leukemia cell line HL-60. The effect of histamine was very rapid and was sustained through a 15-min incubation period at 37°, in the absence of any phosphodiesterase inhibitor. These results are in sharp contrast to the IBMX dependence for histamine-induced cyclic AMP accumulation in human peripheral neutrophils (7). Under the same experimental conditions, histamine produced only an early and transient 2.5-fold increase in cellular cyclic AMP levels in mature granulocytes, whereas stimulation by histamine was markedly sustained and potentiated (10-fold increase) by 0.5 mM IBMX (7). The maximal capacity for cyclic AMP formation after cell surface stimulation by histamine plus IBMX was 7 times higher in HL-60 cells (70 pmoles/10^6 cells) than in circulating neutrophils (10 pmoles/10^6 cells). Similarly, in the absence of IBMX, the non-histamine agonist PGE_1 induced a significant accumulation of cyclic AMP in HL-60 cells (90 pmoles/10^6 cells, i.e., a 50-fold increase over basal levels), whereas cyclic AMP levels were increased only from 1.38 to 8.24 pmoles/10^6 cells in human peripheral neutrophils incubated in the presence of IBMX. Therefore, the major differences between the two cell types were in the basal activity, which was about 7–8 times higher in HL-60 cells incubated in the absence (or in the presence) of IBMX, and in the remarkable ability of the HL-60 cells to generate cyclic AMP after cell surface interaction with histamine or PGE_1—even in absence of any phosphodiesterase inhibitor. Such differences between mature and immature granulocytes might be accomplished under any of the following conditions: (a) lower cyclic AMP phosphodiesterase activities, (b) a higher density of the GTP regulatory protein and/or the catalytic moiety of the cyclase, (c) larger stores of the substrate ATP in HL-60 cells, or

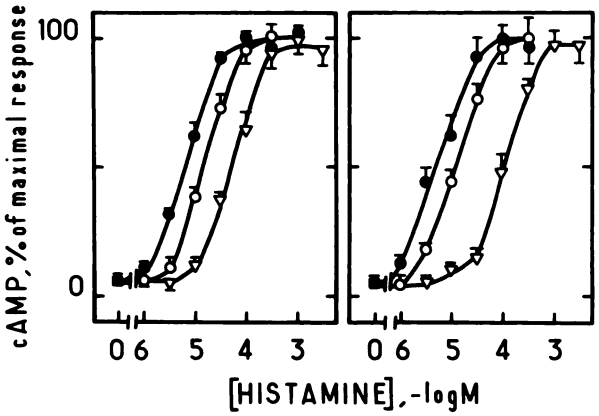

FIG. 6. *Inhibition by H_1 or H_2 receptor antagonists of the histamine-induced cyclic AMP accumulation in HL-60 cells*

Cells were preincubated for 10 min at 37° in the absence of IBMX and incubated for 5 min following the addition of histamine alone or in combination with its antagonists at two different concentrations. In each experiment, results are expressed as the percentage of the maximal cyclic AMP stimulation elicited by histamine (left, 38.2 ± 1.8 pmoles of cyclic AMP per 10^6 cells; right, 43 ± 3.3 pmoles of cyclic AMP per 10^6 cells) in the absence of antagonist. Results are means ± standard error of the mean of results from one experiment performed in triplicate. Three other experiments gave similar results. Basal cyclic AMP levels (■) were measured in HL-60 cells.

Left. K_i values were obtained for cimetidine in the presence of 10^{-6} M (○, $K_i = 0.63 \times 10^{-6}$ M) or 5×10^{-6} M cimetidine (▽, $K_i = 0.62 \times 10^{-6}$ M).

Right. K_i values were obtained for DPH in the presence of 10^{-4} M (○, $K_i = 67 \times 10^{-6}$ M) or 5×10^{-4} M diphenhydramine (▽, $K_i = 30 \times 10^{-6}$ M).

(d) a combination of these three conditions. This matter is currently under investigation in our laboratory. In favor with the first possibility, Smith and Peters (12) have shown that the specific activity of the low K_m cyclic AMP phosphodiesterase shows a 7-fold reduction in neutrophils from patients with chronic granulocytic leukemia as compared with control subjects.

The results obtained with the series of H_1 or H_2 agonists and antagonists identify the histamine receptor in HL-60 cells as being of the H_2 type. The reported inhibition constants for the H_2 receptor antagonist cimetidine ($K_i = 0.65 \times 10^{-6}$ M) and that of the H_1 receptor antagonist DPH ($K_i = 51 \times 10^{-6}$ M) are comparable to the relative affinities of these antagonists ($H_2 > H_1$) for the neutrophil histamine H_2 receptor ($K_i = 0.4$ and 65×10^{-6} M, respectively) (7). In contrast, DPH was approximately 10^3–10^4 times more potent on typical H_1 receptors (13, 14). Our data revealed that the furane derivative ranitidine and the imidazole derivative oxmetidine were 4.7 and 1.6 times more potent, respectively, than the substituted imidazole analogue cimetidine in HL-60 cells. Accordingly, these two H_2 receptor antagonists were 5–15 times more potent than cimetidine at the level of the gastric histamine H_2 receptor *in vivo* (15, 16) as well as *in vitro* (17, 18). On the other hand, the order of potency of the H_1- and H_2-selective agonists on HL-60 cells (impromidine > histamine > 4-MH > AET > PEA) agrees well with their relative potencies on the H_2 receptors evidenced in human neutrophils (7) and in human gastric glands (17), whereas an inverse sequence ($H_1 > H_2$) was found for H_1 receptors (19). Therefore, the lower potencies of the nontautomeric heterocyclic molecules AET and PEA versus the H_2 receptor-selective agonists impromidine and 4-MH ($H_2 > H_1$) further support the H_2-type classification for the histamine receptor in HL-60 cells.

From these results, it is clear that there are no substantial differences between the pharmacological properties of the histamine H_2 receptors in HL-60 cells and in mature granulocytes (7). One exception was the increased affinity and the decreased efficacy of impromidine, which appears to be a partial agonist since it produces only 15–20% of the maximal cyclic AMP response to histamine in HL-60 cells. Indeed, in human circulating mature granulocytes, impromidine achieved the same cyclic AMP production as histamine when tested alone or in combination with histamine (7). Such differences between the potency and the efficacy of impromidine on HL-60 cells and in mature granulocytes may be due to differences in the structural requirements of impromidine-histamine receptor interactions, or to variations in receptor-adenylate cyclase coupling. This suggests that a fewer number of histamine receptors could be occupied or activated by impromidine in HL-60 cells. Accordingly, impromidine antagonized the effect of histamine and produced a parallel displacement of the histamine dose-response curve to the right, suggesting that impromidine was also acting as a competitive antagonist on HL-60 cells. It is also known that several compounds, which include agonists and antagonists of histamine, are potent inhibitors of histamine-*N*-methyltransferase (20, 21), the principal enzyme responsible for histamine degradation in tissues (20). In peripheral leukocytes, histamine methyltransferase is contained exclusively in monocytes, and histaminase (22) in granulocytes. Therefore, destruction or alteration of added compounds could be considered to explain the differences in the potency of impromidine in promyelocytic HL-60 cells and in mature granulocytes. However, it is important to note that the partially agonistic action of impromidine does not exclude the possibility that the cyclic AMP-dependent protein kinases in HL-60 cells (23) may be fully activated by impromidine. Indeed, a marginal increase in cellular cyclic AMP concentration could evoke a subsequent maximal activation of the cyclic nucleotide-dependent enzymes as noted at the level of the gastric histamine H_2 receptor (24). In agreement, impromidine, which was also a partial agonist on H_2 receptors mediating cyclic AMP production in human fundic glands (18) and in the human gastric cancer cell line HGT-1 (25, 26), induced a near-maximal stimulation of gastric acid secretion (27).

In conclusion, our data indicate that the histamine H_2 receptors evidenced in the human promyelocytic leukemia cell line HL-60 exhibit pharmacological characteristics similar to those of the H_2 receptors evidenced in peripheral neutrophils (7). Therefore, those histamine receptors retain their high degree of chemical specificity during myeloid differentiation to mature granulocytes. It should be emphasized also that the histamine- and prostaglandin-sensitive cyclic AMP systems evidenced in HL-60 cells are representative of the biochemical events that are associated with granulocytic functions (28) after terminal myeloid cell differentiation. Similarly, histamine has been shown to elevate cyclic AMP levels via H_2 receptors in fundic glands isolated from human (29) and rat (30) fetuses, before morphological or functional differentiation of the acid-secreting parietal cells. The high cyclic AMP levels measured in leukemic HL-60 cells incubated under basal and stimulated conditions are consistent with data in other cell systems in which sustained elevations of cyclic AMP inhibit cell proliferation (31). Therefore, the presence of cell surface receptors for histamine and prostaglandin as early as the promyelocyte stage during granulopoiesis supports a possible role for these agonists through a cyclic AMP-dependent mechanism (32) on the regulation of proliferation-differentiation of human myeloid progenitor cells. This seems likely because the non-thiourea H_2 antagonist cimetidine might produce a toxic effect at the level of the granulocytic precursors *in vivo* during the treatment of peptic ulcer disease (2, 33) and *in vitro* on formation of bone marrow myeloid colonies (34). This possibility was further sustained by the presence of prostaglandin or histamine H_2 receptors triggering the granulocyte/macrophage precursors (human and murine multipotential hemopoietic stem cells) from the G_0 state into the S-phase of the cell cycle (35, 36).

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