0026-895X/92/020235-07$03.00/0

MOLECULAR PHARMACOLOGY, 42:235–241

Histamine Increases Cytosolic Ca^{2+} in HL-60 Promyelocytes Predominantly via H_2 Receptors with an Unique Agonist/ Antagonist Profile and Induces Functional Differentiation

ROLAND SEIFERT, ARIANE HÖER, INGO SCHWANER, and ARMIN BUSCHAUER

Institut für Pharmakologie, Universitätsklinikum Rudolf Virchow, Freie Universität Berlin, D-1000 Berlin 33, Germany (R.S., A.H., I.S.), and Institut für Pharmazie, Freie Universität Berlin, D-1000 Berlin 33, Germany (A.B.)

Received July 10, 1991; Accepted May 4, 1992

SUMMARY

Histamine H_1 receptors mediate activation of phospholipase C, with subsequent increases in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), and H_2 receptors mediate accumulation of cAMP. HL-60 promyelocytes possess H_2 receptors, but it is not known whether these cells also possess H_1 receptors. We studied the effects of histamine on $[Ca^{2+}]_i$ and the functional importance of histamine receptors in HL-60 promyelocytes. In these cells, histamine and dimaprit increased $[Ca^{2+}]_i$ with EC_{50} values of 15 μM and 30 μM, respectively. Diphenhydramine inhibited the effect of histamine (100 μM) on $[Ca^{2+}]_i$ up to 40%, with an IC_{50} of 100 nM. Famotidine and cimetidine diminished the effect of histamine (100 μM) up to 75%, with IC_{50} values of 85 nM and 300 nM, respectively. Diphenhydramine plus famotidine abolished histamine-induced rises in $[Ca^{2+}]_i$. Impromidine, with an IC_{50} of 100 nM, abolished the effect of histamine (100 μM) on $[Ca^{2+}]_i$. Diphenhydramine, famotidine, cimetidine, and impromidine showed marked noncompetitive antagonism with histamine. Histamine-induced increases in $[Ca^{2+}]_i$ were largely due to influx of Ca^{2+} from the extracellular space. Ca^{2+} influx was inhibited by 1-{β-[3-(4-methoxyphenyl)propoxyl]-4-methoxyphenethyl}-1*H*-imidazole hydrochloride (SK&F 96365). Histamine activated phospholipase C. Histamine induced expression of formyl peptide receptors, which effect was abolished by famotidine. In U-937 promonocytes and in the human erythroleukemia cell lines HEL and K-562, histamine did not induce rises in $[Ca^{2+}]_i$. Our data suggest the following. (i) In HL-60 promyelocytes, histamine increases $[Ca^{2+}]_i$ predominantly via H_2 receptors and to a lesser extent via H_1 receptors. (ii) The agonist/antagonist profile of the H_2 receptor-mediated increases in $[Ca^{2+}]_i$ differs markedly from that for cAMP accumulation, suggesting the involvement of different H_2 receptor subtypes. (iii) In HL-60 promyelocytes, histamine activates nonselective cation channels and induces functional differentiation via H_2 receptors.

HL-60 promyelocytes possess histamine H_2 receptors, which mediate activation of adenylyl cyclase, with subsequent increases in cAMP (1, 2). Dibutyryl-cAMP-differentiated HL-60 cells possess H_1 and H_2 receptors; the former receptors mediate activation of phospholipase C and of nonselective cation channels, resulting in an increase in $[Ca^{2+}]_i$ (3, 4). Stimulation by dimaprit of H_2 receptors in HL-60 promyelocytes results in differentiation towards neutrophils (2). Histamine induces neutrophilic differentiation of HL-60 cells as well, but it is not known whether this occurs via H_1 or H_2 receptors (5). Interestingly, activation of H_1 receptors may result in potentiation of H_2 receptor-mediated cAMP accumulation (6). HL-60 promyelocytes also possess ATP receptors, which mediate activation of phospholipase C and increases in $[Ca^{2+}]_i$ (7–10). Intriguingly, ATP induces functional differentiation of HL-60 cells, as assessed by increased expression of formyl peptide receptors (9).

This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

The findings described above prompted us to study the effects of histamine on $[Ca^{2+}]_i$ and the functional importance of histamine receptors in HL-60 promyelocytes. We report here that histamine increases $[Ca^{2+}]_i$ in HL-60 promyelocytes predominantly via H_2 receptors. We suggest that H_2 receptor-mediated rises in $[Ca^{2+}]_i$ and cAMP involve different H_2 receptor subtypes, and we show that histamine, via H_2 receptors, induces functional differentiation of HL-60 promyelocytes.

Experimental Procedures

Materials. (R)-α-Methylhistamine was a gift from Dr. W. Schunack (Institut für Pharmazie, Freie Universität Berlin, Berlin, Germany). Pertussis toxin was donated by Dr. M. Yajima (Kaken Pharmaceutical, Otsu, Japan). SK&F 96365 was a gift from Dr. D. Arndts (Boehringer Ingelheim, Ingelheim, Germany). *myo*-[2-^{3}H]Inositol (10–20 Ci/mmol) was purchased from Amersham-Buchler (Braunschweig, Germany). Arpromidine, dimaprit, and impromidine were synthesized as described

ABBREVIATIONS: $[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; EGTA, ethylene bis(oxyethylenenitrilo)tetraacetic acid; fMet-Leu-Phe, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; fura-2/AM, fura-2/acetoxymethyl ester; $InsP_3$, inositol trisphosphate; SK&F 96365, 1-{β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl}-1*H*-imidazole hydrochloride; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

(11–13). U-937, HEL, and K-562 cells were purchased from the American Type Culture Collection (Rockville, MD). Fetal calf serum was obtained from GIBCO (Berlin, Germany). Sources of other materials have been described elsewhere (3, 4, 10, 14, 15).

Cell culture. HL-60 promyelocytes were grown in suspension culture in RPMI 1640 medium supplemented with 10% (v/v) horse serum, 1% (v/v) nonessential amino acids, 2 mM L-glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin, in a humidified atmosphere with 7% CO_2 at 37°. For experiments with pertussis toxin-treated cells, HL-60 promyelocytes were treated with pertussis toxin (1 μg/ml) or its vehicle (control) for 24 hr. Thereafter, cells were harvested and experiments were performed. HL-60 promyelocytes were differentiated towards neutrophils upon exposure for 96 hr to histamine (100 μM) (5). U-937, HEL, and K-562 cells were grown in suspension culture in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 1% (v/v) nonessential amino acids, 2 mM L-glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin, in a humidified atmosphere with 5% CO_2 at 37°. Cell viability was assessed by trypan blue dye exclusion.

Measurement of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was determined with the dye fura-2/AM, as described (10), with modifications. Cells were suspended at 1×10^7 cells/ml in a buffer consisting of (in mM) 138 NaCl, 6 KCl, 1 $MgSO_4$, 1 Na_2HPO_4, 5 $NaHCO_3$, 5.5 glucose, and 20 HEPES-NaOH, pH 7.4, supplemented with 0.1% (w/v) bovine serum albumin. Fura-2/AM was added at a concentration of 4 μM, and cells were incubated for 10 min at 37°. Thereafter, cells were diluted with the aforementioned buffer to a concentration of 5×10^6 cells/ml and were incubated for 45 min at 37°. Subsequently, cells were diluted with the aforementioned buffer to a final concentration of 0.5×10^6 cells/ml and were centrifuged at 250 × g for 10 min at 20°. Cells were suspended at 1.0×10^6 cells/ml in the aforementioned buffer and were kept at 20° until measurement of $[Ca^{2+}]_i$. HL-60 promyelocytes were used for up to 4 hr after loading with fura-2/AM (7). Experiments with the other myeloid cell types were completed within 1–1.5 hr. Within these times, basal $[Ca^{2+}]_i$ in myeloid cells did not rise by more than 20–40 nM, and the responsiveness to any of the stimuli studied did not change significantly. Basal $[Ca^{2+}]_i$ in freshly loaded HL-60 promyelocytes was 103 ± 8 nM (mean ± standard deviation; 20 different preparations of HL-60 cells) and did not differ significantly in control and pertussis toxin-treated cells (data not shown). Myeloid cells (1.0×10^6 cells) were suspended in 2 ml of the aforementioned buffer, using acryl fluorescence cuvettes (Sarstedt, Nümbrecht, Germany). Fluorescence was determined at 37°, with constant stirring of the cells at 1×10^3 rpm, using a Ratio II spectrofluorometer (Aminco, Silver Spring, MD). Cells were incubated for 3 min at 37°, in the absence or presence of various substances (e.g., histamine receptor antagonists), before the addition of stimuli; basal fluorescence (basal $[Ca^{2+}]_i$) was measured for 1 min. The excitation and emission wavelengths were 340 and 500 nm, respectively. Basal $[Ca^{2+}]_i$ values and peak $[Ca^{2+}]_i$ values stimulated by agonists were calculated according to eq. 6 given in Ref. 16. Basal $[Ca^{2+}]_i$ values were subtracted from the corresponding peak $[Ca^{2+}]_i$ values, to calculate the increase in $[Ca^{2+}]_i$ induced by a given agonist. Unless stated otherwise, all experiments were performed in the presence of extracellular Ca^{2+} (1 mM $CaCl_2$ added to the buffer 3 min before stimuli). Quantitative comparison of peak $[Ca^{2+}]_i$ values in myeloid cells (e.g., comparison of control cells versus antagonist- or pertussis toxin-treated cells) is based on the responses to stimuli in different aliquots of a given preparation of loaded cells. For the generation of complex concentration-response curves (see Figs. 1–4), the following procedure was adopted. Immediately after an aliquot of cells was challenged with a stimulus, the next aliquot of cells was equilibrated to 37° in a water bath. After 2 min ($[Ca^{2+}]_i$ values in the challenged aliquot of cells had already declined), the fresh aliquot was placed into the fluorometer, to assess basal $[Ca^{2+}]_i$. By this procedure, up to 80 aliquots of cells could be analyzed within 4 hr. Due to the stability in the responsiveness of HL-60 promyelocytes, agonist and/or antagonist concentrations were not randomized, but they were varied in a systematic manner by starting with agonists and/or antagonists at low concentrations.

Labeling of phosphoinositides in HL-60 promyelocytes and measurement of inositol phosphate formation. For labeling of phosphoinositides in HL-60 cells, cells were grown for 48 hr in inositol-free RPMI 1640 medium supplemented with 10% (v/v) horse serum, 1% (v/v) nonessential amino acids, 2 mM L-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, and 2–5 μCi/ml *myo*-[2-^{3}H]inositol, in a humidified atmosphere with 7% CO_2 at 37°. Prelabeled cells were centrifuged at 250 × g for 10 min at 20°. Cells were suspended in the buffer used for the determination of $[Ca^{2+}]_i$. Cells were recentrifuged and suspended in buffer. After another centrifugation of the cells, they were suspended at 1×10^7 cells/ml in buffer. Reactions were performed at 37° in buffer supplemented with 1 mM $CaCl_2$, in a final volume of 200 μl. Reactions were initiated by addition of 100 μl of cells to 100 μl of buffer containing solvent (control) or the stimulus at the desired concentration. Assays did not contain LiCl. Reactions were stopped after 30 sec by addition of 400 μl of a solution consisting of $CHCl_3$, CH_3OH, and concentrated HCl (100:200:1, v/v/v). Thereafter, 125 μl of $CHCl_3$ and 25 μl of H_2O were added to the reaction mixtures. After centrifugation for phase separation, 350 μl of the aqueous phase were loaded on Dowex 1 × 8 columns (0.8 × 2 cm). Inositol phosphates were eluted as described (17). The eluates (8 ml) were mixed with 12 ml of Flow-szint IV scintillation fluid (Camberra Packard, Frankfurt/Main, Germany), and radioactivity was determined by scintillation counting.

Calculations. EC_{50} and IC_{50} values were obtained by graphically analyzing the concentration-response curves shown in Figs. 1–4.

Results

Histamine and dimaprit induce increases in $[Ca^{2+}]_i$ in HL-60 promyelocytes. Histamine increased $[Ca^{2+}]_i$ with an EC_{50} of 15 μM and a maximum at 0.3–1.0 mM (Fig. 1). Dimaprit increased $[Ca^{2+}]_i$ with an EC_{50} of 30 μM and a maximum at 100 μM. The effectiveness of dimaprit (1 mM) to increase $[Ca^{2+}]_i$ amounted to 20% of that of histamine (1 mM). Pertussis toxin ADP-ribosylates heterotrimeric regulatory guanine nucleotide-binding proteins of the G_i family and thereby prevents cell activation by various receptor agonists (4, 7, 10). In HL-60 promyelocytes, however, pertussis toxin treatment did not affect the histamine- and dimaprit-induced rises in $[Ca^{2+}]_i$ (see Fig. 1).

Betahistine (a weak partial H_1 agonist) (6, 18), arpromidine (a potent H_2 agonist and H_1 antagonist) (11), impromidine (a potent H_2 agonist and H_3 antagonist and weak H_1 antagonist) (6, 18), and (R)-α-methylhistamine (a potent H_3 agonist) (18, 19), at concentrations up to 100 μM, did not induce rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes (data not shown). Diphenhydramine (an H_1 antagonist) (6, 18) and cimetidine and famotidine (H_2 antagonists) (18, 20), at up to 100 μM each, did not affect basal $[Ca^{2+}]_i$ or induce a rise in $[Ca^{2+}]_i$ in HL-60 promyelocytes (data not shown). Diphenhydramine, cimetidine, famotidine, and impromidine, at up to 100 μM each, showed no inhibitory effect on rises in $[Ca^{2+}]_i$ induced by ATP (10 μM) (data not shown). The cell-permeant cAMP analogue dibutyryl-cAMP (1 mM) did not induce rises in $[Ca^{2+}]_i$ (data not shown).

H_1 and H_2 antagonists and impromidine inhibit histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes. The effects of diphenhydramine, cimetidine, famotidine, and impromidine on the rises in $[Ca^{2+}]_i$ induced by histamine (100 μM) were studied (Fig. 2). Diphenhydramine reversed stimulation caused by histamine with an IC_{50} of 100 nM and a plateau at 10–100 μM. Diphenhydramine (100 μM) reduced the stimulatory effect of histamine by approximately 40%. Cimetidine and famotidine inhibited the effects of histamine with IC_{50} values of 300 nM and 85 nM, respectively, and both substances at 100 μM diminished the histamine-induced rises in $[Ca^{2+}]_i$ by

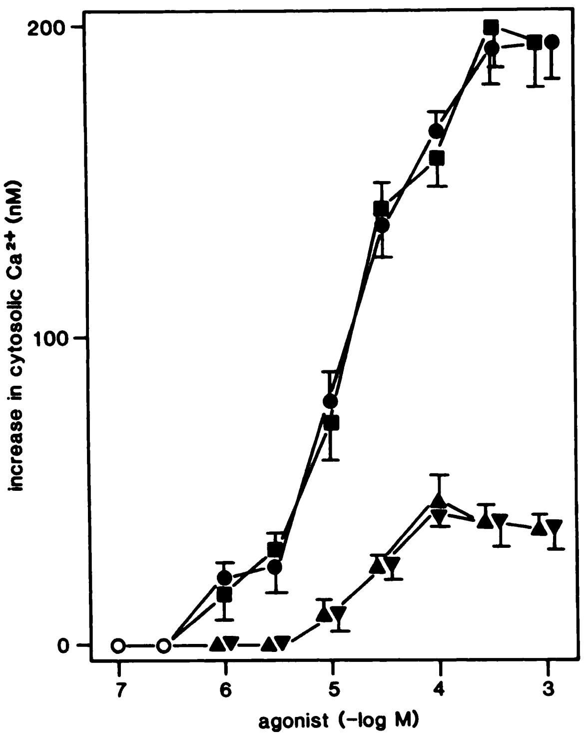

Fig. 1. Concentration-response curves for histamine- and dimaprit-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes and lack of effect of pertussis toxin. HL-60 cells were treated with pertussis toxin (1 μg/ml) or its vehicle (control) for 24 hr. Thereafter, cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by histamine or dimaprit were assessed. ■, Control cells stimulated with histamine; ●, pertussis toxin-treated cells stimulated with histamine; ▲, control cells stimulated with dimaprit; ▼, pertussis toxin-treated cells stimulated with dimaprit. ○, Substances at the designated concentrations did not increase $[Ca^{2+}]_i$. Data shown are the means ± standard deviations of four experiments performed with different preparations of HL-60 cells.

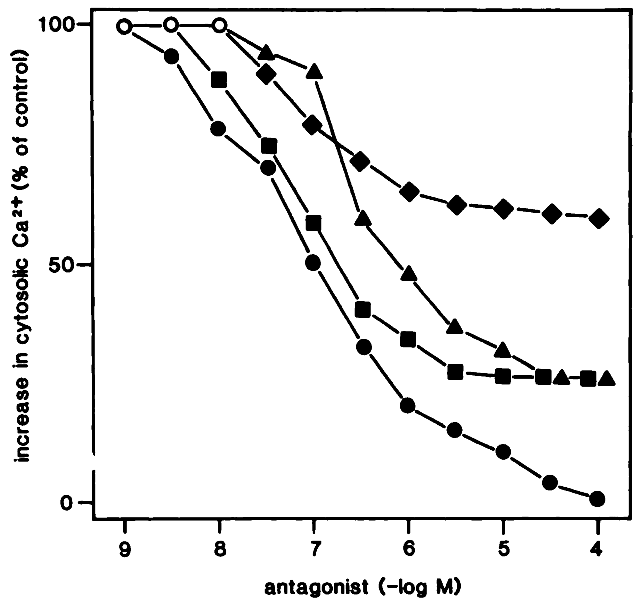

Fig. 2. Concentration-response curves for the inhibitory effects of diphenhydramine, cimetidine, famotidine, and impromidine on histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes. HL-60 cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by histamine at a fixed concentration (100 μM), in the presence of antagonists at various concentrations, were assessed. In the absence of antagonists, histamine (100 μM) increased $[Ca^{2+}]_i$ by 194 ± 14 nM (mean ± standard deviation of five experiments performed with different preparations of HL-60 cells). Antagonists were added to cells 3 min before histamine. ♦, Diphenhydramine; ▲, cimetidine; ■, famotidine; ●, impromidine. ○, Antagonists at the designated concentrations did not show an inhibitory effect on histamine-induced rises in $[Ca^{2+}]_i$. Data shown are the means of five experiments performed with different preparations of HL-60 cells. The standard deviation values of the data generally amounted to <10% of the means.

about 75%. Impromidine antagonized the effects of histamine with an IC_{50} of 100 nM, and inhibition was complete at 100 μM. Famotidine (100 μM) abolished the stimulatory effect of dimaprit (100 μM) on $[Ca^{2+}]_i$, whereas diphenhydramine (100 μM) was ineffective (data not shown).

Fig. 3 shows concentration-response curves for histamine in the absence or presence of diphenhydramine, famotidine, or a combination of both antagonists at a high concentration (100 μM each). Diphenhydramine reduced the effectiveness of histamine to increase $[Ca^{2+}]_i$, without changing its EC_{50}. Famotidine increased the EC_{50} for histamine by about 4-fold and greatly reduced its effectiveness. Marked reductions in the effectiveness of histamine (1 μM to 3 mM) to induce rises in $[Ca^{2+}]_i$ were also observed with famotidine at 100 nM, 1 μM, and 10 μM (data not shown). The combination of diphenhydramine plus famotidine (100 μM each) abolished the stimulatory effects of histamine, at concentrations as high as 3 mM, on $[Ca^{2+}]_i$ (see Fig. 3).

The effects of impromidine and cimetidine, at increasing fixed concentrations, on the concentration-response curve for histamine were assessed (Fig. 4). Impromidine, in a concentration-dependent manner, decreased the effectiveness of histamine to induce rises in $[Ca^{2+}]_i$. Impromidine at up to 1 μM did not substantially increase the EC_{50} for histamine. Impromidine at 10 and 100 μM increased the EC_{50} for histamine by about 6- and 30-fold, respectively. Cimetidine greatly depressed the concentration-response curve for histamine and slightly increased its EC_{50}.

Histamine activates nonselective cation channels in HL-60 promyelocytes. Histamine induced transient increases in $[Ca^{2+}]_i$ (Fig. 5). Histamine-induced rises in $[Ca^{2+}]_i$ depended largely on Ca^{2+} influx, inasmuch as in the absence of extracellular Ca^{2+} the magnitude and duration of rises in $[Ca^{2+}]_i$ were greatly reduced. SK&F 96365 is a blocker of nonselective cation channels and inhibits receptor agonist-induced Ca^{2+} influx in neutrophils and in dibutyryl-cAMP-differentiated HL-60 cells (4, 21). SK&F 96365 (10 μM) substantially diminished histamine-induced Ca^{2+} influx in HL-60 promyelocytes (see Fig. 5). SK&F 96365 (30 μM) abolished histamine-induced Ca^{2+} influx without affecting Ca^{2+} mobilization from intracellular stores (data not shown). Thus, histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes were largely due to Ca^{2+} influx through nonselective cation channels.

Histamine activates phosphoinositide degradation in HL-60 promyelocytes. A small part of the histamine-induced rise in $[Ca^{2+}]_i$ in HL-60 promyelocytes was due to mobilization of Ca^{2+} from intracellular stores (see Fig. 5). Because Ca^{2+} mobilization is preceeded by formation of $InsP_3$ (7), the effects of histamine on phosphoinositide degradation were studied. In agreement with the small effect of histamine on Ca^{2+} mobilization, histamine slightly stimulated formation of $InsP_3$ (Table 1).

Effects of ATP and histamine on $[Ca^{2+}]_i$ in various human myeloid cell types. ATP induces increases in $[Ca^{2+}]_i$ in HL-60 promyelocytes and U-937 promonocytes and induces differentiation of these cells (7–9). Therefore, we studied the effects of histamine and ATP on $[Ca^{2+}]_i$ in various

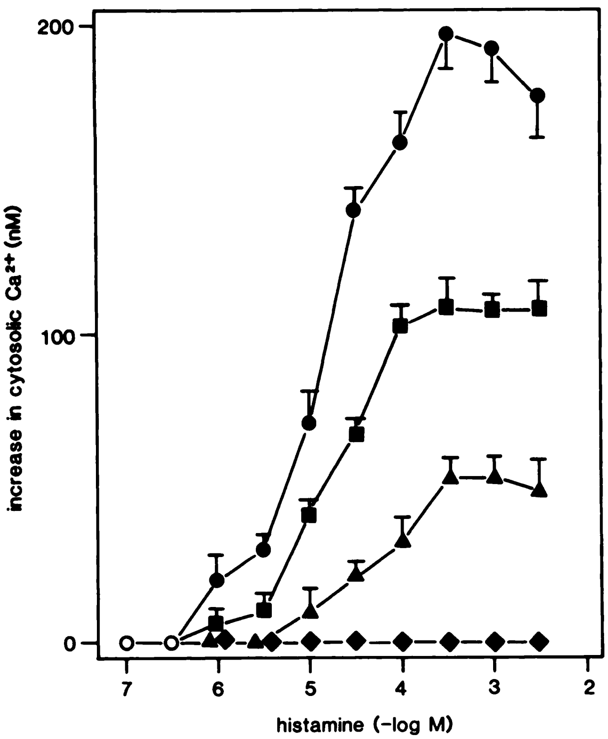

Fig. 3. Inhibition by diphenhydramine, famotidine, or a combination of both antagonists of histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes. HL-60 cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by histamine at various concentrations, in the absence or presence of antagonists at fixed concentrations (100 μM each), were assessed. Antagonists or solvent (control) were added to cells 3 min before histamine. ●, Control; ■, diphenhydramine; ▲, famotidine; ♦, diphenhydramine plus famotidine; ○, histamine at the designated concentrations did not increase $[Ca^{2+}]_i$. Data shown are the means ± standard deviations of four experiments performed with different preparations of HL-60 cells.

human myeloid cell lines, i.e., in HL-60 promyelocytes, in U-937 promonocytes (8, 9, 22), and in the human erythroleukemia cell lines HEL (23) and K-562 (24). ATP was much more effective than histamine in activating phosphoinositide degradation and inducing rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes (Table 2; see also Table 1). ATP also effectively increased $[Ca^{2+}]_i$ in U-937 promonocytes and in HEL cells, whereas in K-562 cells ATP did not induce rises in $[Ca^{2+}]_i$. Unlike ATP, histamine did not show stimulatory effects on $[Ca^{2+}]_i$ in U-937 promonocytes and in HEL cells, and it also did not induce rises in $[Ca^{2+}]_i$ in K-562 cells.

Histamine induces functional differentiation of HL-60 cells, which is inhibited by famotidine. In order to assess the functional importance of histamine receptors in HL-60 promyelocytes, the effects of histamine, famotidine, and diphenhydramine on differentiation were studied. Differentiation of HL-60 promyelocytes results in increased expression of formyl peptide receptors, which process is readily monitored by the increased effectiveness of fMet-Leu-Phe to induce rises in $[Ca^{2+}]_i$ (9, 10, 15). The responsiveness to fMet-Leu-Phe of HL-60 cells treated for 96 hr with histamine (100 μM) was substantially greater than that of HL-60 promyelocytes (Table 3). Famotidine abolished the effect of histamine, whereas diphenhydramine showed no effect. Famotidine and diphenhydramine *per se* did not induce changes in the effectiveness of fMet-Leu-Phe to induce rises in $[Ca^{2+}]_i$ in HL-60 cells. In order to evaluate the contribution of Ca^{2+} influx to histamine-induced differentiation, SK&F 96365 (30 μM) was added to the culture medium. SK&F 96365 was cytotoxic, i.e., after 48 hr >95% of the cells had lost viability (data not shown). Similarily, chelation of Ca^{2+} by EGTA in the culture medium was cytotoxic (data not shown).

Discussion

H_2 receptors mediate a large portion of histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes. In HL-60 promyelocytes, histamine induces cAMP accumulation via H_2 receptors (1). Additionally, histamine activates phospholipase C-catalyzed $InsP_3$ formation, Ca^{2+} mobilization from intracellular stores, and Ca^{2+} influx through nonselective cation channels in these cells (see Fig. 5 and Table 1). It is unlikely that histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes were due to histamine-induced rises in cAMP, inasmuch as a cell-permeant analogue of cAMP did not increase $[Ca^{2+}]_i$. Surprisingly, the effects of histamine on $[Ca^{2+}]_i$ in HL-60 promyelocytes are mediated largely via H_2 receptors and not via H_1 receptors, as suggested by several findings. First, H_2 antagonists (cimetidine and famotidine) partially inhibited the stimulatory effects of histamine (see Figs. 2–4). Similarly to other systems, famotidine was a more potent H_2 antagonist than cimetidine in HL-60 promyelocytes (see Fig. 2) (18, 20). Additionally, an

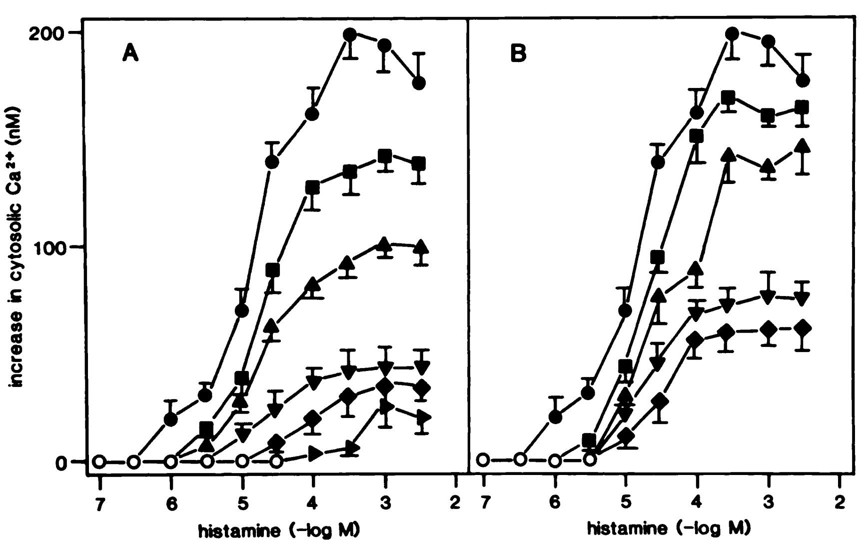

Fig. 4. Inhibition by impromidine and cimetidine of histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes. HL-60 cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by histamine at various concentrations, in the absence or presence of antagonists at fixed concentrations, were assessed. Antagonists or solvent (control) were added to cells 3 min before histamine. A, ●, Control; ■, impromidine (10 nM); ▲, impromidine (100 nM); ▼, impromidine (1 μM); ♦, impromidine (10 μM); ▶, impromidine (100 μM). B, ●, Control; ■, cimetidine (100 nM); ▲, cimetidine (300 nM); ▼, cimetidine (1 μM); ♦, cimetidine (3 μM). ○, Histamine at the designated concentrations did not increase $[Ca^{2+}]_i$. Data shown are the means ± standard deviations of four experiments performed with different preparations of HL-60 cells.

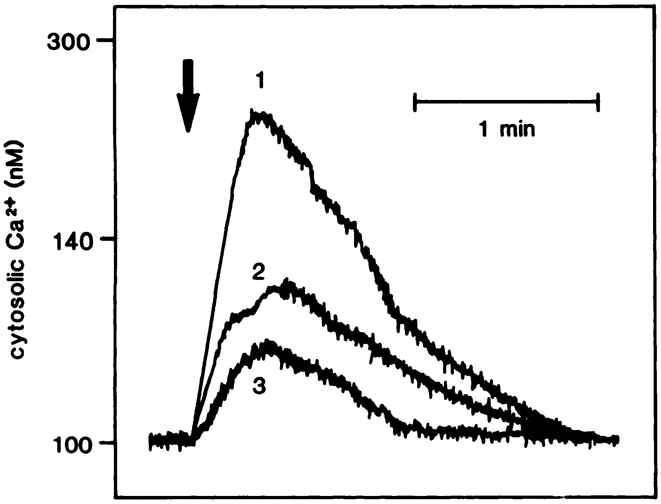

Fig. 5. Effect of extracellular Ca^{2+} and of SK&F 96365 on histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes. HL-60 cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by histamine (100 μM) were assessed. *Arrow*, addition of histamine. Three minutes before histamine, various substances were added to cells. *Trace 1*, $CaCl_2$ (1 mM); *trace 2*, $CaCl_2$ (1 mM) plus SK&F 96365 (10 μM); *trace 3*, EGTA (1 mM) without added $CaCl_2$. Superimposed original fluorescence tracings are shown. Similar results were obtained in three experiments performed with different preparations of HL-60 cells.

TABLE 1

Effects of histamine and ATP on phosphoinositide degradation in HL-60 promyelocytes

HL-60 cells were cultured for 48 hr in inositol-free RPMI medium supplemented with *myo*-[2-^{3}H]inositol, under the conditions described in Experimental Procedures. Thereafter, labeled cells were washed. HL-60 cells (1 × 10^6 cells in a volume of 200 μl) were exposed to solvent (control), histamine, or ATP (100 μM each) for 30 sec at 37°, in the buffer used for the determination of $[Ca^{2+}]_i$ supplemented with 1 mM $CaCl_2$. Stopping of the reactions, extraction of inositol phosphates, and separation of inositol phosphates on Dowex 1 × 8 columns were performed as described in Experimental Procedures. Data shown are the means ± standard deviations of assay triplicates. Similar results were obtained in eight experiments performed with different preparations of HL-60 cells.

Stimulus	Inositol phosphates[a]		
	InsP	$InsP_2$	$InsP_3$
		dpm	
Solvent (control)	1042 ± 179	948 ± 49	391 ± 40
Histamine	885 ± 52	925 ± 129	534 ± 32
ATP	1353 ± 36	3021 ± 198	980 ± 73

[a] InsP, inositol monophosphate; $InsP_2$, inositol bisphosphate.

TABLE 2

Effects of ATP and histamine on $[Ca^{2+}]_i$ in various human myeloid cell types

HL-60 promyelocytes, U-937 promonocytes, HEL cells, and K-562 cells were cultured as described in Experimental Procedures. Cells were loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by ATP and histamine (100 μM each) were assessed. Basal $[Ca^{2+}]_i$ in the cell types studied ranged from 100 to 230 nM. Data shown are the means ± standard deviations of assay triplicates. Similar results were obtained in up to nine experiments performed with different preparations of myeloid cells.

Cell type	Increase in $[Ca^{2+}]_i$	
	ATP	Histamine
	nM	
HL-60 promyelocytes	1084 ± 103	213 ± 20
U-937 promonocytes	664 ± 134	0
HEL cells	360 ± 57	0
K-562 cells	0	0

TABLE 3

Effects of histamine, diphenhydramine, and famotidine on fMet-Leu-Phe-induced rises in $[Ca^{2+}]_i$ in HL-60 cells

HL-60 promyelocytes were cultured for 96 hr with histamine (100 μM), to induce neutrophilic differentiation. For comparison, HL-60 promyelocytes were cultured for 96 hr in the absence of histamine. In addition, cell cultures contained solvent (control) or histamine receptor antagonists at the indicated concentrations. HL-60 cells were loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by fMet-Leu-Phe (1 μM) were assessed. Basal $[Ca^{2+}]_i$ in the six groups of cells ranged from 100 to 120 nM. Data shown are the means ± standard deviations of assay quadruplicates. Similar results were obtained in three experiments with different preparations of HL-60 cells.

Addition	Increase in $[Ca^{2+}]_i$	
	HL-60 promyelocytes	HL-60 cells (histamine)
	nM	
Solvent (control)	105 ± 8	447 ± 26
Diphenhydramine (10 μM)	109 ± 12	468 ± 34
Famotidine (10 μM)	98 ± 10	110 ± 12

H_2 agonist (dimaprit) increased $[Ca^{2+}]_i$ in HL-60 promyelocytes to some extent (see Fig. 1), whereas a full H_3 agonist and a partial H_1 agonist did not. Moreover, famotidine but not diphenhydramine antagonized the stimulatory effects of dimaprit.

The lack of inhibitory effect of pertussis toxin on histamine- and dimaprit-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes is also in accordance with the aforementioned assumption, because H_2 receptors couple to the pertussis toxin-insensitive guanine nucleotide-binding protein G_s (see Fig. 1) (6, 18, 25). Whether H_2 receptors additionally couple to one or more of the recently cloned pertussis toxin-insensitive guanine nucleotide-binding proteins, e.g., G_q or G_{16}, to activate phospholipase C and/or nonselective cation channels, remains to be determined (26). Interestingly, in dimethyl sulfoxide-differentiated HL-60 cells, H_2 receptors mediate increases in $[Ca^{2+}]_i$ via pertussis toxin-insensitive guanine nucleotide-binding proteins as well (27). Moreover, H_2 receptor-mediated rises in $[Ca^{2+}]_i$ were reported for gastric parietal cells (28). Thus, stimulation of rises in $[Ca^{2+}]_i$ via H_2 receptors is not restricted to HL-60 promyelocytes but may be a more general phenomenon.

Does a H_2 receptor subtype mediate the histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes? The EC_{50} values for various H_2 receptor-mediated responses to histamine in human neutrophils and HL-60 promyelocytes are similar, as are the maximally effective concentrations of histamine (see Fig. 1) (1, 3, 14, 22, 27, 29, 30). However, the H_2 receptor-mediated increases in $[Ca^{2+}]_i$ in HL-60 promyelocytes show pharmacological properties that are quite different from those for inhibition of fMet-Leu-Phe-induced superoxide formation in human neutrophils, for cAMP accumulation in neutrophils and HL-60 promyelocytes, and for positive inotropy and/or chronotropy in the guina pig atrium (standard model for the characterization of H_2 receptors) (1–3, 6, 11, 14, 18, 20). Impromidine, in addition to being an H_2 agonist, is a potent H_3 and weak H_1 antagonist (6, 18, 19). Interestingly, impromidine is a partial agonist at H_2 receptors in various systems, including H_2 receptors in HL-60 promyelocytes mediating increases in cAMP (1, 22, 31–33). In contrast to other systems, impromidine is not an agonist with respect to increases in $[Ca^{2+}]_i$ in HL-60 promyelocytes (1, 3, 11, 29). Instead, impromidine reversed histamine-induced rises in $[Ca^{2+}]_i$ with a potency similar to that of famotidine (see Fig. 2). Impromidine acted as a mixed competitive/noncompetitive antagonist and, unlike famotidine, cimetidine, and diphenhydramine, impromidine abolished the rise in $[Ca^{2+}]_i$ induced by histamine at 100 μM (see Figs. 2 and 4). It is unlikely that histamine acted via H_3 receptors, because (R)-α-methylhistamine, a potent and selective H_3 agonist (6, 18, 19), did not increase $[Ca^{2+}]_i$. Additionally, dimaprit, which shows H_3 antagonistic effects (19), increased $[Ca^{2+}]_i$ in HL-60 promyelocytes (see Fig. 1). Moreover, the stimulatory effects of dimaprit on $[Ca^{2+}]_i$ were inhibited by famotidine. Thus, it appears that impromidine acted as a dual H_1/H_2 antagonist to inhibit histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes (see also below).

Similarly to impromidine, the structurally related guanidine arpromidine (11) failed to induce rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes, and dimaprit was about 2-fold less potent and 5-fold less effective than histamine in this regard (see Fig. 1). By comparison, arpromidine is a full agonist with respect to inhibition of fMet-Leu-Phe-induced superoxide formation in neutrophils and positive inotropy in the guinea pig atrium (11, 14). Additionally, dimaprit is approximately as potent and as effective as histamine in increasing cAMP in HL-60 promyelocytes (2). The failure of H_2 agonists to mimic the effects of histamine is not without precedence in the literature (34).

In human neutrophils and HL-60 promyelocytes, cimetidine acts as a competitive antagonist to reverse histamine- and dimaprit-induced cAMP accumulations, and famotidine is a competitive antagonist of histamine- and arpromidine-induced inhibition of superoxide formation (1–3, 14, 29). With respect to histamine-induced increases in $[Ca^{2+}]_i$ in HL-60 promyelocytes, famotidine, cimetidine, and impromidine showed marked noncompetitive antagonism (see Figs. 3 and 4). It is, however, unlikely that these substances inhibited rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes in a nonspecific manner, because they did not inhibit ATP-induced rises in $[Ca^{2+}]_i$. In other systems, famotidine shows noncompetitive antagonism versus dimaprit, but cimetidine does not (18, 20). Moreover, famotidine was only 3.5-fold more potent than cimetidine in reversing histamine-induced increases in $[Ca^{2+}]_i$ in HL-60 promyelocytes, but in other systems famotidine is at least 10-fold more potent than cimetidine (see Fig. 2) (18, 20). Taken together, the agonist/antagonist profiles for H_2 receptor-mediated increases in cAMP and in $[Ca^{2+}]_i$ in HL-60 promyelocytes are quite different. Additionally, there are substantial pharmacological differences between H_2 receptor-mediated increases in $[Ca^{2+}]_i$ in HL-60 promyelocytes and responses in other systems. These data suggest that histamine mediates increases in cAMP and in $[Ca^{2+}]_i$ in HL-60 promyelocytes through different receptor subtypes.

What may be the function of H_2 receptor-mediated increases in $[Ca^{2+}]_i$ in HL-60 promyelocytes? Histamine induces neutrophilic differentiation of HL-60 promyelocytes, but it is unknown through which receptor subtype histamine acts (5). We found that histamine induced expression of formyl peptide receptors in HL-60 cells (see Table 3). This effect of histamine was mediated via H_2 receptors, inasmuch as it was abolished by famotidine (see Table 3). These data support the notion that H_2 receptors play a role in neutrophilic differentiation of myeloid progenitor cells, and they are in good agreement with the clinical finding that long term treatment with H_2 antagonists may lead to neutropenia or agranulocytosis (1, 2, 35).

In HL-60 promyelocytes, histamine induces parallel increases in cAMP and in $[Ca^{2+}]_i$ (see Figs. 1–5) (1). Ca^{2+} influx through nonselective cation channels is required for histamine-induced differentiation, inasmuch as blockade of these channels by SK&F 96365 was cytotoxic and prevented differentiation. H_1 receptor-mediated rises in $[Ca^{2+}]_i$ may potentiate H_2 receptor-mediated cAMP accumulation (6). Similarily, H_2 receptor-mediated rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes may potentiate H_2 receptor-mediated cAMP accumulation. Histamine is much more effective at inducing rises in cAMP in HL-60 promyelocytes than in human neutrophils (1, 29). The fact that histamine does not induce rises in $[Ca^{2+}]_i$ in human neutrophils may account, at least in part, for this difference between the two cell types (1, 4, 29). By analogy to histamine, prostaglandin E_1 is another inducer of differentiation of HL-60 cells (36). The effectiveness of prostaglandin E_1 to induce rises in cAMP is greater in HL-60 promyelocytes than in human neutrophils, and prostaglandin E_1 induces rises in $[Ca^{2+}]_i$ in the former cells but not in the latter (1, 29, 15, 37). Thus, histamine- and prostaglandin E_1-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes may amplify cAMP accumulation, resulting in the induction of differentiation.

Not only H_2 receptors but also ATP receptors mediate functional differentiation of human myeloid cells (see Table 3) (2, 9). Similar to histamine, ATP activates phospholipase C and induces rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes (see Tables 1 and 2) (7, 10, 15). However, stimulation of ATP receptors does not result in cAMP accumulation (7–9). The Ca^{2+} ionophore A23187 induces differentiation of HL-60 promyelocytes as well (38, 39). Thus, an increase in $[Ca^{2+}]_i$ may provide a sufficient signal for the induction of differentiation, independently of cAMP accumulation. H_2 receptor-mediated rises in $[Ca^{2+}]_i$ do not play a role in the induction of differentiation of U-937, HEL, and K-562 cells, inasmuch as histamine did not increase $[Ca^{2+}]_i$ in these cells (see Table 2). In contrast, ATP increased $[Ca^{2+}]_i$ in U-937 promonocytes and in HEL cells. Additionally, ATP was substantially more effective than histamine in activating phosphoinositide degradation and inducing rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes (see Tables 1 and 2) (7, 10, 15). These data suggest that ATP and H_2 receptor-mediated rises in $[Ca^{2+}]_i$ play different roles in the regulation of myeloid differentiation processes.

H_1 receptors mediate a small portion of histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes. Evidence in support of the notion that H_1 receptors mediated a small part of the histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes comes from the finding that famotidine failed to reverse completely the stimulation caused by histamine (100 μM) (see Fig. 2). However, famotidine (100 μM) plus diphenhydramine (100 μM) abolished the effects of histamine on $[Ca^{2+}]_i$ (see Fig. 3). With histamine at 100 μM, the H_2 antagonist-insensitive portion of rises in $[Ca^{2+}]_i$ amounted to about 25%, presumably reflecting the H_1 receptor-mediated responses (see Fig. 2). The fact that diphenhydramine (100 μM) inhibited the responses towards histamine (100 μM) to a greater extent than was expected from the data obtained with cimetidine and famotidine may be explained by H_2 antagonistic properties of diphenhydramine at the high concentration used (see Fig. 2) (1, 29). The IC_{50} for diphenhydramine on rises in $[Ca^{2+}]_i$ induced by histamine (100 μM) in HL-60 promyelocytes is in agreement with that for dibutyryl-cAMP-differentiated HL-60 cells, and in both cell types diphenhydramine showed marked noncompetitive antagonism (see Figs. 2 and 3) (4). Moreover, the data obtained with impromidine support the view that H_1 receptors mediated, to a small extent, histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 promyelocytes (see Figs. 2 and 4) (4, 6, 18). Apparently, H_1 receptor-mediated increases in $[Ca^{2+}]_i$ *per se* were too small to induce differentiation of HL-60 promyelocytes, inasmuch as diphenhydramine did not block stimulation by histamine of formyl peptide receptor expression (see Table 3).

In conclusion, histamine increases $[Ca^{2+}]_i$ in HL-60 promyelocytes predominantly via H_2 receptors and, to a lesser extent, via H_1 receptors. H_2 receptor-mediated rises in $[Ca^{2+}]_i$ and cAMP may be mediated through different H_2 receptor subtypes. Activation by histamine of H_2 receptors in HL-60 promyelocytes results in activation of nonselective cation channels and in functional differentiation.

Acknowledgments

The authors are grateful to Dr. G. Schultz for helpful suggestions, to M. Bigalke, E. Glaß, E. Kanth, and I. Reinsch for expert technical assistance, to J. Fischer for performing some preliminary experiments of this study, and to Dr. K. Wenzel-Seifert for providing software to calculate $[Ca^{2+}]_i$.

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Send reprint requests to: Dr. Roland Seifert, Institut für Pharmakologie, Universitätsklinikum Rudolf Virchow, Freie Universität Berlin, Thielallee 69/73, D-1000 Berlin 33, Germany.