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Histamine Increases Cytosolic Ca^{2+} in Dibutyryl-cAMP-Differentiated HL-60 Cells via H_1 Receptors and Is an Incomplete Secretagogue

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SUMMARY

Human neutrophils and dibutyryl-cAMP (Bt_2cAMP)-differentiated HL-60 cells possess receptors for the chemotactic peptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMet-Leu-Phe), which mediate activation of phospholipase C, with subsequent increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and activation of specific cell functions. In many cell types, histamine, via H_1 receptors, activates phospholipase C, but it is unknown whether neutrophilic cells possess functional H_1 receptors. We compared the effects of histamine with those of fMet-Leu-Phe on activation of these cells. In Bt_2cAMP-differentiated HL-60 cells, substances increased $[Ca^{2+}]_i$ in the effectiveness order fMet-Leu-Phe > histamine > betahistine. Pertussis toxin diminished fMet-Leu-Phe-induced rises in $[Ca^{2+}]_i$ to a greater extent than those induced by histamine. H_1 but not H_2 antagonists inhibited histamine- and betahistine-induced rises in $[Ca^{2+}]_i$. fMet-Leu-Phe and histamine activated phospholipase C and increased $[Ca^{2+}]_i$ through release of Ca^{2+} from intracellular stores and sustained influx of Ca^{2+} from the extracellular space. The substances also induced Mn^{2+} influx. Ca^{2+} and Mn^{2+} influxes were inhibited by 1-{β-[3-(4-methoxyphenyl)propoxyl]-4-methoxyphenethyl}-1*H*-imidazole hydrochloride (SK&F 96365). The stimulatory effects of histamine on $[Ca^{2+}]_i$ were more sensitive to inhibition by 4β-phorbol 12-myristate 13-acetate than were those of fMet-Leu-Phe. Unlike fMet-Leu-Phe, histamine did not activate superoxide anion formation, release of β-glucuronidase, and tyrosine phosphorylation. In neutrophils, histamine and betahistine did not induce rises in $[Ca^{2+}]_i$. Our data show that (i) in Bt_2cAMP-differentiated HL-60 cells, histamine increases $[Ca^{2+}]_i$ via H_1 receptors coupled to pertussis toxin-sensitive and possibly, pertussis toxin-insensitive heterotrimeric regulatory guanine nucleotide-binding proteins, (ii) histamine activates nonselective cation channels, and (iii) unlike fMet-Leu-Phe, histamine is an incomplete secretagogue.

The intercellular signal molecule histamine mediates its effects through H_1, H_2, and H_3 receptors (1, 2). Stimulation of H_1 receptors results in the activation of phospholipase C, which catalyzes phosphoinositide degradation to diacylglycerol and $InsP_3$; diacylglycerol activates protein kinase C, and $InsP_3$ mobilizes Ca^{2+} from intracellular stores, resulting in an increase in $[Ca^{2+}]_i$ (1–3). H_1 Receptors interact with pertussis toxin-sensitive or pertussis toxin-insensitive G proteins, depending on the cell type studied (3–7). H_2 Receptors interact with the G protein G_s, leading to the activation of adenylyl cyclase, with subsequent increase in cAMP (1–3).

Neutrophilic cells, i.e., neutrophils and differentiated HL-60 cells, possess receptors for the chemotactic peptide fMet-Leu-Phe, which couple to pertussis toxin-sensitive G proteins (8–20). fMet-Leu-Phe induces activation of phospholipase C, phosphorylation of tyrosine, and opening of nonselective cation channels, which mediate sustained influx of Ca^{2+} from the extracellular space (8–10, 20–22). Exposure of these cells to fMet-Leu-Phe culminates in activation of NADPH oxidase-catalyzed O_2^- formation and in release of β-glucuronidase from azurophilic granules (8–10, 13, 23, 24). Neutrophilic cells and HL-60 promyelocytes possess H_2 receptors; activation of these receptors leads to inhibition of fMet-Leu-Phe-stimulated O_2^- formation and induction of cell differentiation, respectively (25–31). It is, however, unknown whether human myeloid cells possess functional H_1 receptors, although the results of binding studies suggested the presence of such receptors (32–34). By analogy to formyl peptide receptors, activation of H_1 receptors in myeloid cells would be expected to result in increases in

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ABBREVIATIONS: $InsP_3$, inositol trisphosphate; Bt_2cAMP, dibutyryl-cAMP; $[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; EGTA, ethylene bis(oxyethylenenitrilo)tetraacetic acid; G protein, heterotrimeric regulatory guanine nucleotide-binding protein; fMet-Leu-Phe, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; fura-2/AM, fura-2/acetoxymethyl ester; O_2^-, superoxide anion; PDD, 4α-phorbol-12,13-didecanoate; PMA, 4β-phorbol 12-myristate 13-acetate; SK&F 96365, 1-{β-[3-(4-methoxyphenyl)propoxyl]-4-methoxyphenethyl}-1*H*-imidazole hydrochloride; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

$[Ca^{2+}]_i$ and stimulation of O_2^- formation. We and others could not obtain evidence to support the assumption that H_1 receptors play a part in the activation of O_2^- formation in human neutrophils (27, 29).

HL-60 cells differentiated towards neutrophils with Bt_2cAMP are a widely used model system to study signal transduction processes in human myeloid cells (11–16, 18). Among other receptors, these cells very effectively express functional ATP and UTP receptors (12–15). These receptors couple to both pertussis toxin-sensitive and pertussis toxin-insensitive G proteins, leading to activation of phospholipase C (12–15, 18). In Bt_2cAMP-differentiated HL-60 cells, ATP and UTP *per se* stimulate O_2^- formation and β-glucuronidase release, whereas in human neutrophils they do not (12, 13, 24).

All these findings prompted us to address the question of whether Bt_2cAMP-differentiated HL-60 cells provide a suitable model system to study H_1 receptors in human myeloid cells. We show here that histamine, via H_1 receptors, increases $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells and that histamine is an incomplete secretagogue.

Experimental Procedures

Materials. Pertussis toxin was donated by Dr. M. Yajima (Kaken Pharmaceutical, Otsu, Japan). SK&F 96365 was a gift of Dr. D. Arndts (Boehringer Ingelheim, Ingelheim, Germany). Clemastine, chlorpheniramine, diphenhydramine, and famotidine were purchased from Sigma Chemie (Taufkirchen, Germany). *myo*-[2-^{3}H]Inositol (10–20 Ci/mmol) was purchased from Amersham-Buchler (Braunschweig, Germany). Monoclonal antiphosphotyrosine antibody PY-20 (IgG2B) was from ICN (Meckenheim, Germany). Sources of other materials have been described elsewhere (12, 13, 24, 29–31, 35).

Cell culture. HL-60 cells 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°. To induce differentiation, HL-60 cells were seeded at 1×10^6 cells/ml and were cultured for 48 hr with 0.2 mM Bt_2cAMP (12, 13).

Pertussis toxin treatment of HL-60 cells. Differentiation of HL-60 cells was induced as described above. Twenty-four hours before experiments, pertussis toxin (1 μg/ml) or its vehicle (control) was added to the cell cultures. Under these conditions, pertussis toxin functionally inactivated >98% of the cellular content of pertussis toxin-sensitive G proteins (15, 23).

Isolation of human neutrophils. Neutrophils were isolated from buffy coats from the local blood bank or from fresh blood of individual healthy volunteers (24, 29, 30). Cell preparations consisted of >95% viable neutrophils, as revealed by trypan blue dye exclusion and Pappenheim-stained smears.

Measurement of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was determined with the dye fura-2/AM, as described (13), with modifications. HL-60 cells or neutrophils 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 $250 \times g$ 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 cells were used for up to 4 hr after loading with fura-2/AM (14). HL-60 cells or neutrophils (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. 36. 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 stimulus. Influx of Mn^{2+} into HL-60 cells was measured by quenching of fura-2 fluorescence, as described (21). 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). Basal $[Ca^{2+}]_i$ in freshly loaded HL-60 cells was 117 ± 18 nM (mean ± standard deviation; 25 different preparations of cells). Basal $[Ca^{2+}]_i$ in control cells and in pertussis toxin-treated HL-60 cells did not differ significantly (data not shown). Basal $[Ca^{2+}]_i$ in human neutrophils was 102 ± 21 nM (mean ± standard deviation; seven different preparations of cells). Within 4 hr after loading of the HL-60 cells, basal $[Ca^{2+}]_i$ did not rise by more than 20 nM, and the responsiveness to any of the stimuli studied did not change significantly during that time. In addition, there was little variation in the responsiveness to the various stimuli using different batches of HL-60 cells. Quantitative comparison of peak $[Ca^{2+}]_i$ values in HL-60 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 HL-60 cells. For the generation of complex concentration-response curves (see Figs. 1–4), the following procedure was adopted. Immediately after an aliquot of HL-60 cells was challenged with a stimulus, the next aliquot of HL-60 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 h. Due to the stability in the responsiveness of cells, 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 cells and measurement of inositol phosphate formation. For labeling of phosphoinositides in HL-60 cells, cells were seeded at a density of 1×10^6 cells/ml 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, 0.2 mM Bt_2cAMP, and 2–5 μCi/ml *myo*-[2-^{3}H]inositol, in a humidified atmosphere with 7% CO_2 at 37°. Cells were cultured for 48 hr. Prelabeled cells were centrifuged at $250 \times g$ for 10 min at 20°. Cells were suspended in the buffer used for the determination of $[Ca^{2+}]_i$. Cells were recentrifuged and were 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 the aforementioned 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 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 (37). 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.

Assay for O_2^- formation. O_2^- formation was monitored by continuous measurement of ferricytochrome *c* reduction that was inhibitable by superoxide dismutase, using an Uvikon 810 dual-beam spectrophotometer (Kontron, Eching, Germany) (12, 13). Reaction mixtures (0.5

ml) contained 2.5 × 10^6 HL-60 cells, 100 μM ferricytochrome *c*, and a buffer consisting of (in mM) 138 NaCl, 6 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5.5 glucose, and 20 HEPES-NaOH, pH 7.4. Reaction mixtures were incubated for 3 min at 37° before the addition of stimuli. The absolute amounts of O_2^- generated were calculated.

β-Glucuronidase release. Enzyme release was assessed as described (13). Briefly, HL-60 cells (5.0 × 10^6 cells in 0.5 ml) were suspended in the buffer used for the determination of O_2^- formation. Cells were incubated for 5 min at 37° in the presence of cytochalasin B (5 μg/ml) before the addition of stimuli. Reactions were conducted for 10 min and were terminated by placement of the tubes on ice. Reaction mixtures were centrifuged at 1000 × *g* for 10 min at 4°. The determinations of the activities of lactate dehydrogenase and β-glucuronidase in supernatant fluids of reaction mixtures and in cell lysates were performed as described (24). Lactate dehydrogenase release from cells generally amounted to <5% of cellular content (data not shown).

Tyrosine phosphorylation. Tyrosine phosphorylation of proteins was performed as described (35). In brief, HL-60 cells (1.0 × 10^6 cells in 40 μl) were suspended in the buffer used for the determination of O_2^- formation. Cells were incubated for 3 min at 37° before the addition of stimuli (20 μl). Reactions were terminated after 1 min by addition of 30 μl of lysis buffer [6% (w/v) sodium dodecyl sulfate, 18% (v/v) 2-mercaptoethanol, 30% (v/v) glycerol, 1 mM Na_3VO_4, and a trace amount of Bromophenol blue dye, in 0.2 M Tris·HCl, pH 7.5]. Samples were immediately incubated for 5 min at 95°. Thereafter, samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on gels containing 9% (w/v) acrylamide. The separated proteins were then transferred onto nitrocellulose filters, which were saturated with ovalbumin. Filters were incubated for 1 hr with 1 μg/ml monoclonal antiphosphotyrosine antibodies. Subsequently, filters were incubated with a goat anti-mouse IgG-peroxidase conjugate. Bound antibodies were detected by the enhanced chemiluminescence Western blotting detection system (Amersham, Braunschweig, Germany). Phosphotyrosine-containing proteins were detected by exposure to medical X-ray films (autoluminography).

Calculations. EC_{50} and IC_{50} values were obtained by graphically analyzing the concentration-response curves shown in Figs. 1–4. The pA_2 for diphenhydramine versus histamine (see Fig. 2B) was calculated as described (38, 39).

Results

Pertussis toxin partially inhibits histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 cells. Concentration-response curves for the effects of fMet-Leu-Phe and histamine on $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells are shown in Fig. 1. fMet-Leu-Phe increased $[Ca^{2+}]_i$ with an EC_{50} of 3 nM and a maximum at 100 nM. Pertussis toxin decreased the effectiveness of fMet-Leu-Phe (1 μM) by about 70% and reduced its potency by almost 10-fold. Histamine increased $[Ca^{2+}]_i$ in a concentration-dependent manner as well; the EC_{50} was 5 μM and a plateau was reached at 30–100 μM. The effectiveness of histamine (100 μM) to increase $[Ca^{2+}]_i$ was 30% of that of fMet-Leu-Phe (1 μM). Pertussis toxin inhibited the effect of histamine (100 μM) on $[Ca^{2+}]_i$ by 35%.

H_1 antagonists inhibit histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 cells. Fig. 2A shows the effects of various H_1 antagonists on the concentration-response curve for histamine. Clemastine (a potent H_1 antagonist) (1, 2) at 100 nM abolished the stimulatory effects of histamine on $[Ca^{2+}]_i$. Chlorpheniramine, another potent H_1 antagonist (1, 2), at 100 nM reduced the effectiveness of histamine to increase $[Ca^{2+}]_i$ by >70% and increased its EC_{50} by about 2-fold. Impromidine (a potent H_2 agonist and H_3 antagonist and weak H_1 antagonist) (1, 2) at 100 μM increased the EC_{50} for histamine by almost 10-fold and reduced its effectiveness by about 20%. Diphenhydramine (1, 2, 40), another H_1 antagonist, at increasing fixed concentrations shifted the concentration-response curve for histamine to the right and substantially reduced the effectiveness of the agonist (Fig. 2B). The pA_2 for diphenhydramine was 7.9. Clemastine, chlorpheniramine, and diphenhydramine inhibited rises in $[Ca^{2+}]_i$ induced by histamine (100 μM) with IC_{50} values of 3, 20, and 100 nM, respectively (Fig. 3). Impromidine was much less effective than the former substances, and famotidine (a potent H_2 antagonist) (41) was ineffective. Diphenhydramine, chlorpheniramine, clemastine, famotidine,

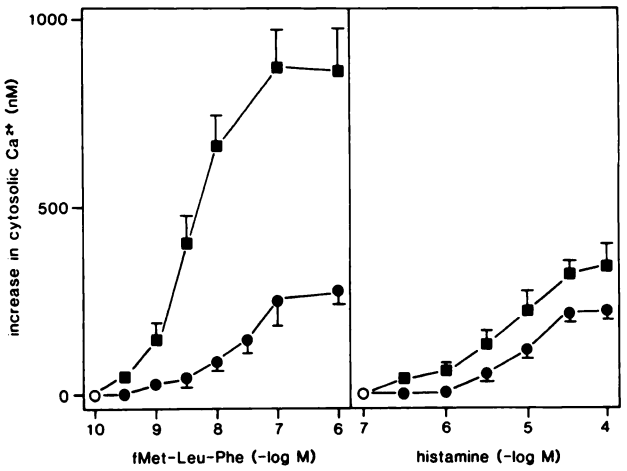

Fig. 1. Concentration-response curves for fMet-Leu-Phe- and histamine-induced rises in $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells and effect of pertussis toxin. Bt_2cAMP-differentiated HL-60 cells were treated with pertussis toxin (1 μg/ml) or its vehicle (control) for 24 hr. Thereafter, cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by various substances were assessed. ■, Control cells; ●, pertussis toxin-treated cells. ○, fMet-Leu-Phe and histamine at the designated concentrations did not increase $[Ca^{2+}]_i$. Data shown are the means ± standard deviations of six experiments performed with different preparations of HL-60 cells.

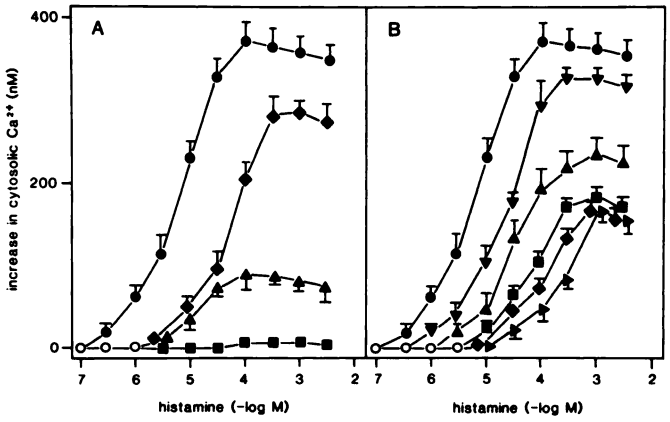

Fig. 2. Inhibition by chlorpheniramine, clemastine, diphenhydramine, and impromidine of histamine-induced rises in $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells. HL-60 cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by histamine at various concentrations, in the absence or presence of antagonists at fixed concentrations, were assessed. Antagonist or solvent (control) was added to cells 3 min before histamine. A, ●, Control; ♦, impromidine (100 μM); ▲, chlorpheniramine (100 nM); ■, clemastine (100 nM). B, ●, Control; ▼, diphenhydramine (30 nM); ▲, diphenhydramine (100 nM); ■, diphenhydramine (300 nM); ♦, diphenhydramine (1 μM); ▶, diphenhydramine (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.

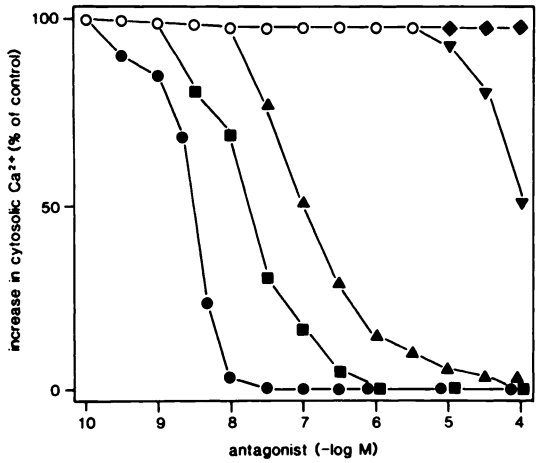

Fig. 3. Concentration-response curves for the inhibitory effects of clemastine, chlorpheniramine, diphenhydramine, impromidine, and famotidine on histamine-induced rises in $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells. HL-60 cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ 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 340 ± 28 nM (mean ± standard deviation of four experiments performed with different preparations of HL-60 cells). Antagonists were added to cells 3 min before histamine. ●, Clemastine; ■, chlorpheniramine; ▲, diphenhydramine; ▼, impromidine; ♦, famotidine. ○, Antagonists at the indicated concentrations did not show an inhibitory effect on histamine-induced rises in $[Ca^{2+}]_i$. Data shown are the means of four experiments performed with different preparations of HL-60 cells. The standard deviation values of the data generally amounted to <10% of the means.

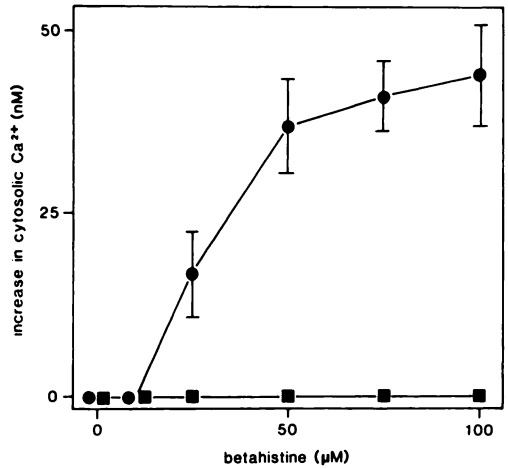

Fig. 4. Concentration-response curve for betahistine-induced rises in $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells and inhibition of the effect of betahistine by clemastine. Bt_2cAMP-differentiated HL-60 cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by betahistine at various concentrations, in the absence or presence of clemastine, were assessed. Clemastine or solvent (control) was added to cells 3 min before betahistine. ●, Control; ■, clemastine (100 nM). Data shown are the means ± standard deviations of three experiments performed with different preparations of HL-60 cells.

and impromidine, at up to 100 μM, did not affect rises in $[Ca^{2+}]_i$ induced by fMet-Leu-Phe (10 nM) (data not shown).

Betahistine is a partial agonist to induce rises in $[Ca^{2+}]_i$ in HL-60 cells. Betahistine (a weak partial H_1 agonist) (1, 2) increased $[Ca^{2+}]_i$ with an EC_{50} of 30 μM and a maximum at 100 μM (Fig. 4). The effectiveness of betahistine to induce rises in $[Ca^{2+}]_i$ amounted to <15% of that of histamine (100 μM) (see Figs. 2 and 4). Clemastine (100 nM) abolished the effects of betahistine (see Fig. 4), whereas famotidine at up to 100 μM was ineffective (data not shown). In contrast to betahistine, dimaprit and arpromidine (H_2 agonists) (1, 2, 42) and (*R*)-α-methylhistamine (an H_3 agonist) (1, 2), at up to 100 μM, did not induce rises in $[Ca^{2+}]_i$ (data not shown).

Histamine activates nonselective cation channels in HL-60 cells. In the presence of extracellular Ca^{2+}, fMet-Leu-Phe caused a rapid and large increase in $[Ca^{2+}]_i$, which returned to basal values within 15–20 min (Fig. 5); data shown only for up to 5 min). In comparison with fMet-Leu-Phe, histamine induced a considerably lower and more transient increase in $[Ca^{2+}]_i$, which was terminated within 3–5 min. In the absence of extracellular Ca^{2+}, the magnitude and duration of rises in $[Ca^{2+}]_i$ induced by both substances were greatly reduced, suggesting that fMet-Leu-Phe and histamine increased $[Ca^{2+}]_i$ predominantly through influx of Ca^{2+} from the extracellular space. The imidazole derivative SK&F 96365 is a blocker of nonselective cation channels and inhibits fMet-Leu-Phe-stimulated influxes of Ca^{2+} and Mn^{2+} in human neutrophils (22). In Bt_2cAMP-differentiated HL-60 cells, SK&F 96365 (10 μM) substantially reduced fMet-Leu-Phe- and histamine-induced Ca^{2+} influx (see Fig. 5). In contrast, SK&F 96365 did not inhibit mobilization of Ca^{2+} from intracellular stores.

Histamine-stimulated Mn^{2+} influx in HL-60 cells was measured by quenching of fura-2 fluorescence. At an excitation wavelength of 340 nm, fluorescence is increased by Ca^{2+} and is reduced by Mn^{2+}; at an excitation wavelength of 360 nm, fluorescence is quenched by Mn^{2+} but is insensitive to changes in $[Ca^{2+}]_i$ (21). At both excitation wavelengths, Mn^{2+} induced a slow decrease in fluorescence, indicating basal Mn^{2+} influx (Fig. 6). At an excitation wavelength of 340 nm but not 360 nm, histamine (100 μM) transiently increased fluorescence,

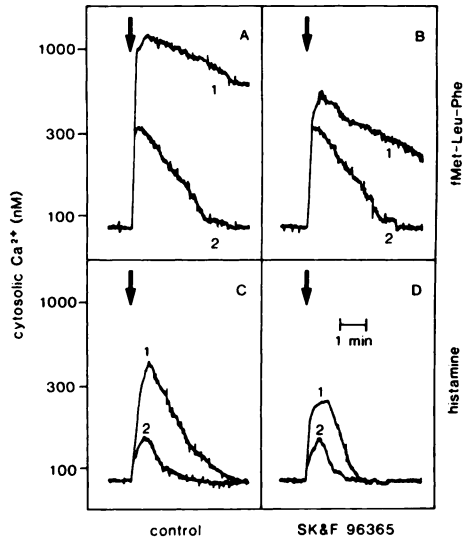

Fig. 5. Effects of extracellular Ca^{2+} and of SK&F 96365 on fMet-Leu-Phe- and histamine-induced rises in $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells. HL-60 cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by fMet-Leu-Phe (100 nM) (A and B) and histamine (100 μM) (C and D) were assessed. *Arrows*, addition of stimuli. Three minutes before stimuli, solvent (control) (A and C) or SK&F 96365 (10 μM) (B and D) was added to cells. *Trace 1*, presence of $CaCl_2$ (1 mM); *trace 2*, presence of 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.

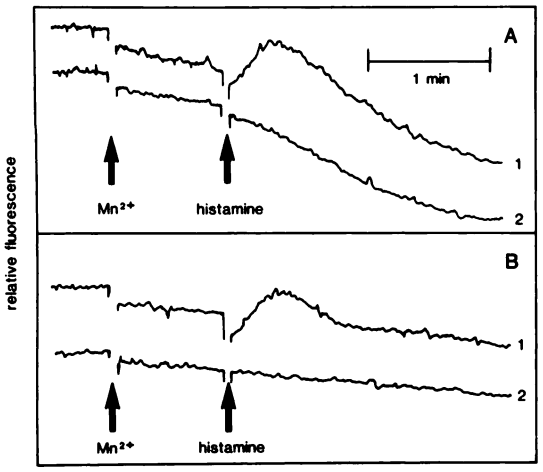

Fig. 6. Effects of histamine on Mn^{2+} influx in Bt_2cAMP-differentiated HL-60 cells. Cells were harvested and loaded with fura-2/AM, and fura-2 fluorescence was monitored at an excitation wavelength of 340 nm (*trace 1*) or 360 nm (*trace 2*). The emission wavelength was 500 nm. Experiments were performed in the absence of added $CaCl_2$. *Arrows*, addition of $MnCl_2$ and histamine (100 μM each). A, Experiments performed in the absence of SK&F 96365 (control); B, experiments performed in the presence of SK&F 96365 (10 μM). SK&F 96365 or solvent (control) was added to cells 3 min before $MnCl_2$. 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 fMet-Leu-Phe and histamine on phosphoinositide degradation in Bt_2cAMP-differentiated HL-60 cells

HL-60 cells were cultured for 48 hr in inositol-free RPMI medium supplemented with *myo*-[2-^{3}H]inositol and Bt_2cAMP, 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), fMet-Leu-Phe (1 μM), or histamine (100 μM) 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 10 experiments performed with different preparations of HL-60 cells.

Stimulus	Inositol phosphates[a]		
	InsP	$InsP_2$	$InsP_3$
	dpm		
Solvent (control)	789 ± 47	705 ± 46	159 ± 40
fMet-Leu-Phe	918 ± 165	1442 ± 40	407 ± 193
Histamine	925 ± 138	838 ± 169	192 ± 45

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

reflecting release of Ca^{2+} from intracellular stores. At excitation wavelengths of 340 and 360 nm, histamine substantially increased the rate of basal fluorescence quenching, reflecting Mn^{2+} influx. SK&F 96365 blocked histamine-induced Mn^{2+} influx but not Ca^{2+} mobilization from intracellular stores. Qualitatively the same results were obtained with fMet-Leu-Phe as stimulus (data not shown). Thus, histamine activates nonselective cation channels, as does fMet-Leu-Phe (see Figs. 5 and 6) (21, 22).

Histamine activates phospholipase C in HL-60 cells. Because small parts of fMet-Leu-Phe- and histamine-induced rises in $[Ca^{2+}]_i$ were due to mobilization of Ca^{2+} from intracellular stores (see Fig. 5), the effects of these substances on phosphoinositide degradation were studied. fMet-Leu-Phe substantially stimulated inositol phosphate formation, in particular the formation of inositol bisphosphate and $InsP_3$ (Table 1). In comparison with fMet-Leu-Phe, histamine only slightly stimulated formation of inositol phosphates.

PMA differentially inhibits fMet-Leu-Phe- and histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 cells. Brief exposure of neutrophilic cells to PMA, an activator of protein kinase C, inhibits fMet-Leu-Phe-induced phosphoinositide degradation and rises in $[Ca^{2+}]_i$, through uncoupling of receptors from G proteins and uncoupling of G proteins from effectors (9, 10, 14, 43). Treatment of HL-60 cells with PMA resulted in substantially diminished stimulatory effects of fMet-Leu-Phe on $[Ca^{2+}]_i$ (Fig. 7). Partial resistance to inhibition by PMA of fMet-Leu-Phe-induced rises in $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells was also reported in another study (14). In contrast, PMA abolished the stimulatory effects of histamine on $[Ca^{2+}]_i$ (see Fig. 7). PDD, which does not activate protein kinase C, did not affect fMet-Leu-Phe- or histamine-induced rises in $[Ca^{2+}]_i$. These data indicate that the inhibitory effects of PMA on rises in $[Ca^{2+}]_i$ were mediated via protein kinase C.

Histamine does not stimulate O_2^- formation, β-glucuronidase release, and tyrosine phosphorylation in HL-60 cells. In order to answer the question of whether histamine-induced rises in $[Ca^{2+}]_i$ are linked to activation of specific cell functions, the effects of histamine on O_2^- formation and β-glucuronidase release were studied. fMet-Leu-Phe effectively activated O_2^- formation and β-glucuronidase release in HL-60 cells (Table 2). In contrast, histamine was devoid of any stimulatory effects. The lack of effect of histamine was also apparent in the presence of famotidine, i.e., when possible inhibitory effects of histamine, via H_2 receptors, on O_2^- formation and β-glucuronidase release were blocked (see Table 2) (27, 29, 30).

fMet-Leu-Phe-induced O_2^- formation is potentiated by ATP and UTP (12, 13, 24). However, histamine and betahistine (0.1 and 1 mM each) did not potentiate O_2^- formation induced by fMet-Leu-Phe (30 nM and 1 μM) in Bt_2cAMP-differentiated HL-60 cells, regardless of the presence or absence of famotidine (10 μM) (data not shown). Similarly, histamine failed to potentiate fMet-Leu-Phe-induced β-glucuronidase release in

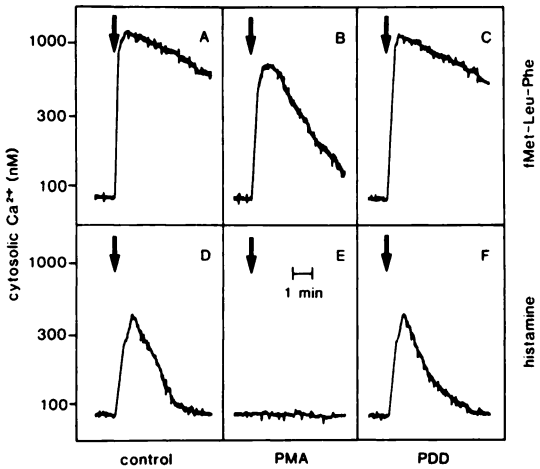

Fig. 7. Effects of PMA and PDD on fMet-Leu-Phe- and histamine-induced rises in $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells. HL-60 cells were harvested and loaded with fura-2/AM, and the increases in $[Ca^{2+}]_i$ induced by fMet-Leu-Phe (100 nM) (A–C) and histamine (100 μM) (D–F) were assessed. *Arrows*, addition of stimuli. Three minutes before stimuli, solvent (control) (A and D), PMA (100 ng/ml) (B and E), or PDD (100 ng/ml) (C and F) was added to cells. Superimposed original fluorescence tracings are shown. Similar results were obtained in three experiments performed with different preparations of HL-60 cells.

TABLE 2

Effects of fMet-Leu-Phe and histamine on O_2^- formation and β-glucuronidase release in Bt_2cAMP-differentiated HL-60 cells

O_2^- formation and β-glucuronidase release in Bt_2cAMP-differentiated HL-60 cells were assessed in the presence of various stimuli. Famotidine was added to cells 3 min before histamine when O_2^- formation was assessed and 5 min before histamine when β-glucuronidase release was studied. Cytochalasin B (5 μg/ml) was added to cells 5 min before stimuli when β-glucuronidase release was determined. For further experimental details, see Experimental Procedures. Data shown are the means ± standard deviations of assay triplicates. Similar results were obtained in three experiments with different preparations of HL-60 cells.

Stimulus	O_2^- formation	β-Glucuronidase release
	nmol/10⁶ cells	*% of cellular content*
Solvent (control)	0	5.2 ± 0.5
fMet-Leu-Phe (1 μM)	6.5 ± 0.8	26.3 ± 1.3
Histamine (100 μM)	0	5.1 ± 0.2
Histamine (1 mM)	0	4.9 ± 0.4
Histamine (100 μM) + famotidine (10 μM)	0	5.3 ± 0.6

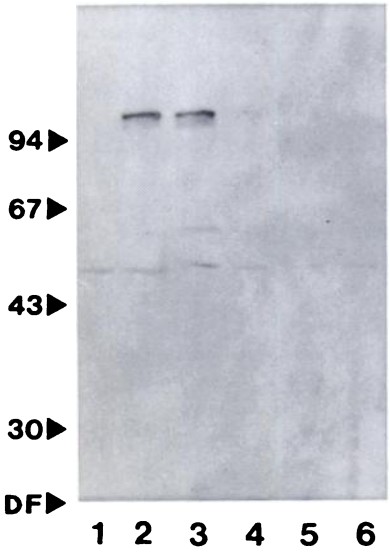

Fig. 8. Effects of fMet-Leu-Phe and histamine on tyrosine phosphorylation in Bt_2cAMP-differentiated HL-60 cells. Cells (1.0×10^6/tube) were incubated with various stimuli for 1 min. Famotidine was added to cells 3 min before histamine. Proteins were analyzed as described in Experimental Procedures. The autoluminogram of a blot is shown. *Lane 1*, solvent (control); *lane 2*, fMet-Leu-Phe (1 μM); *lane 3*, fMet-Leu-Phe (10 μM); *lane 4*, histamine (100 μM); *lane 5*, histamine (1 mM); *lane 6*, histamine (100 μM) plus famotidine (10 μM). *Numbers on the left*, molecular masses of marker proteins (kDa); *DF*, dye front. Similar results were obtained in three experiments performed with different preparations of HL-60 cells.

Bt_2cAMP-differentiated HL-60 cells under various experimental conditions, whereas ATP and UTP synergize with fMet-Leu-Phe (data not shown) (13, 24).

Tyrosine phosphorylation is an important signal for the activation of O_2^- formation by fMet-Leu-Phe (10, 20). Thus, the question arose of whether the lack of stimulation by histamine of O_2^- formation was due to a lack of activation of tyrosine phosphorylation. fMet-Leu-Phe stimulated tyrosine phosphorylation of 100/110-kDa proteins in HL-60 cells (Fig. 8). In contrast, histamine failed to stimulate tyrosine phosphorylation, in the absence or presence of famotidine.

Histamine and betahistine do not induce rises in $[Ca^{2+}]_i$ in neutrophils. Finally, the effects of fMet-Leu-Phe, histamine, and betahistine on $[Ca^{2+}]_i$ in human neutrophils were studied. As was the case for Bt_2cAMP-differentiated HL-60 cells, fMet-Leu-Phe (1 μM) induced large increases in $[Ca^{2+}]_i$ in human neutrophils (data not shown) (see Fig. 1). However, histamine and betahistine (1 μM to 1 mM each) consistently failed to reveal any stimulatory effect on $[Ca^{2+}]_i$ in seven preparations of human neutrophils (data not shown).

Discussion

Functional H_1 receptors coupled to pertussis toxin-sensitive G proteins are present in Bt_2cAMP-differentiated HL-60 cells. Our data suggest that, in Bt_2cAMP-differentiated HL-60 cells, H_1 receptors mediate increases in $[Ca^{2+}]_i$. This assumption is supported by several findings. Most importantly, histamine- and betahistine-induced rises in $[Ca^{2+}]_i$ were inhibited by H_1 but not by H_2 antagonists (see Figs. 2–4). Additionally, the pA_2 for diphenhydramine versus histamine in Bt_2cAMP-differentiated HL-60 cells is in agreement with that obtained for other systems (see Fig. 2) (1, 40). The potency order of clemastine > chlorpheniramine > diphenhydramine ≫ impromidine, to inhibit the effects of histamine on $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells, is in accordance with the classification of an H_1 receptor as well (see Figs. 2 and 3) (1, 2, 40). Moreover, noncompetitive antagonism of clemastine, chlorpheniramine, and diphenhydramine with histamine at H_1 receptors is documented in the literature (see Fig. 2) (1, 40). The lack of effect of H_1 antagonists on fMet-Leu-Phe-induced rises in $[Ca^{2+}]_i$ argues against nonspecific effects of these substances. Similarly to other systems, betahistine is a weak partial H_1 agonist in Bt_2cAMP-differentiated HL-60 cells, and H_2 and H_3 agonists failed to induce rises in $[Ca^{2+}]_i$ (see Figs. 2 and 4) (1, 2).

As in other cell types (1–7), H_1 receptors in Bt_2cAMP-differentiated HL-60 cells mediate activation of phospholipase C and increases in $[Ca^{2+}]_i$ (see Fig. 1 and Table 1). The finding that histamine only marginally activated phosphoinositide degradation fits well with the fact that, in the absence of extracellular Ca^{2+}, histamine induced only small increases in $[Ca^{2+}]_i$ (see Fig. 5 and Table 1). This increase presumably reflects $InsP_3$-mediated mobilization of Ca^{2+} from intracellular stores. The greatest part of histamine-induced rises in $[Ca^{2+}]_i$ in HL-60 cells was attributable to activation of nonselective cation channels (see Figs. 5 and 6). Activation of cation channels via H_1 receptors was also reported for other systems (2, 44).

H_1 receptor-mediated increases in $[Ca^{2+}]_i$ in HL-60 cells were partially inhibited by pretreatment with pertussis toxin (see Fig. 1). These data indicate that, in these cells, H_1 receptors interact with G proteins of the G_i family, as do formyl peptide receptors (8–10). Interestingly, H_1 receptor-mediated increases in $[Ca^{2+}]_i$ in HL-60 cells were more resistant to inhibition by pertussis toxin than were those induced by fMet-Leu-Phe (see Fig. 1). Thus, by analogy to ATP and UTP receptors, H_1 receptors in HL-60 cells may interact with pertussis toxin-sensitive and pertussis toxin-insensitive G proteins (12–15).

Histamine is an incomplete secretagogue. In HL-60 cells, fMet-Leu-Phe and histamine activate phospholipase C and nonselective cation channels, resulting in increases in $[Ca^{2+}]_i$ (see Figs. 5 and 6 and Table 1). There are, however, differences between fMet-Leu-Phe and histamine, i.e., fMet-Leu-Phe is substantially more effective than histamine in activating phospholipase C and inducing rises in $[Ca^{2+}]_i$ (see Fig. 1 and Table 1). Unlike fMet-Leu-Phe, histamine did not stimulate O_2^- formation, β-glucuronidase release, and tyrosine phos-

phorylation (see Fig. 8 and Table 2). These data suggest that even maximal stimulation of H_1 receptors did not result in the generation of sufficiently high levels of intracellular signals to induce activation of specific cell functions, i.e., O_2^- formation and enzyme release. There are differences in the pertussis toxin sensitivity of fMet-Leu-Phe- and histamine-induced rises in $[Ca^{2+}]_i$ (see Fig. 1). Moreover, fMet-Leu-Phe- and histamine-induced rises in $[Ca^{2+}]_i$ were differentially inhibited by PMA (see Fig. 7). These data point to differences in the interactions of H_1 and formyl peptide receptors with G proteins and in the subsequent interaction of G proteins with effectors. Taken together, fMet-Leu-Phe and histamine are functionally nonequivalent, and histamine is an incomplete secretagogue.

In addition to histamine, ATP, UTP, and leukotriene B_4 are incomplete secretagogues (12, 13, 23, 24, 45). Moreover, interaction of H_1, ATP, and leukotriene B_4 receptors with G proteins differs from the interaction of formyl peptide receptors with G proteins (see Figs. 1 and 7) (12–15, 17). However, leukotriene B_4, ATP, and UTP on one hand and histamine on the other hand are functionally nonequivalent. This view is supported by the findings that functional leukotriene B_4 receptors and ATP and UTP receptors are present not only in Bt_2cAMP-differentiated HL-60 cells but also in human neutrophils (9, 10, 12–15, 18, 24). By comparison, there is no evidence for the existence of functional H_1 receptors in neutrophils, as assessed by measurement of $[Ca^{2+}]_i$. Furthermore, leukotriene B_4, ATP, and UTP activate O_2^- formation to at least a small degree or enhance the stimulatory effects of fMet-Leu-Phe on O_2^- formation (12, 13, 23, 24). In contrast, histamine is devoid of any stimulatory effect on O_2^- formation in neutrophils and in Bt_2cAMP-differentiated HL-60 cells (see Table 3) (27, 29).

What may be the regulatory importance of H_1 receptors in HL-60 cells?. In addition to Bt_2cAMP-differentiated HL-60 cells, functional H_1 receptors are present in HL-60 promyelocytes, but there is no evidence to support the assumption that H_1 receptors play a role in the induction of differentiation of HL-60 cells (31). However, the absence of functional H_1 receptors in human neutrophils may point to an as yet unknown role of these receptors in the regulation of differentiation and/or proliferation (31). Interestingly, expression of H_1 receptors in cultured smooth muscle cells is also differentiation dependent (46). In addition, H_1 receptors may be involved in the regulation of proliferation of human fibroblasts, carcinoma cells, and melanoma cells (47, 48).

The possibility cannot be excluded that H_1 receptors in HL-60 cells are of no regulatory importance at all. It should be kept in mind that HL-60 cells are not normal myeloid cells but were derived from a patient with acute promyelocytic leukemia (49). Thus, the expression of functional H_1 receptors in Bt_2cAMP-differentiated HL-60 cells could be a tumor cell-associated abnormality in signal transduction, rather than a phenomenon that occurs in normal neutrophils. Abnormalities in signal transduction processes in Bt_2cAMP-differentiated HL-60 cells, in comparison with neutrophils, are not without precedence. First, ATP and UTP *per se* activate O_2^- formation and β-glucuronidase release in Bt_2cAMP-differentiated HL-60 cells but not in human neutrophils (12, 13, 24). Second, fMet-Leu-Phe induces β-glucuronidase release in Bt_2cAMP-differentiated HL-60 cells in the absence of cytochalasin B but not in human neutrophils (13, 23, 24). Third, PMA stimulates β-glucuronidase release in Bt_2cAMP-differentiated HL-60 cells but not in neutrophils (13, 50). Finally, Bt_2cAMP-differentiated HL-60 cells fail to express a cytosolic component of NADPH oxidase that is normally present in human neutrophils (51). Thus, caution must be exerted when results obtained with Bt_2cAMP-differentiated HL-60 cells are extrapolated to human neutrophils.

In conclusion, histamine increases $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells via H_1 receptors coupled to pertussis toxin-sensitive G proteins and, possibly, to pertussis toxin-insensitive G proteins. Histamine is an incomplete secretagogue, inasmuch as it does not stimulate O_2^- formation and β-glucuronidase release. Regardless of the regulatory importance of H_1 receptors in Bt_2cAMP-differentiated HL-60 cells, this system provides a suitable model to study the pharmacology of H_1 receptors.

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