Histamine Increases Cytosolic Ca\(^{2+}\) in HL-60 Promyelocytes Predominantly via H\(_{2}\) Receptors with an Unique Agonist/Agonist Profile and Induces Functional Differentiation

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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 \(\mu\)M and 30 \(\mu\)M, respectively. Diphenhydramine inhibited the effect of histamine (100 \(\mu\)M) on [Ca\(^{2+}\)]\(_i\), up to 40\%, with an IC\(_{50}\) of 100 \(n\)M. Famotidine and cimetidine diminished the effect of histamine (100 \(\mu\)M) up to 75\%, with IC\(_{50}\) values of 85 \(n\)M and 300 \(n\)M, respectively. Diphenhydramine plus famotidine abolished histamine-induced rises in [Ca\(^{2+}\)]\(_i\). Impromidine, with an IC\(_{50}\) of 100 \(n\)M, abolished the effect of histamine (100 \(\mu\)M) 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-[\(\beta\)-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1H-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 adenyl 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).

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)-\(\alpha\)-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. Arndt (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; InsP3, inositol trisphosphate; SK&F 96365, 1-\(\beta\)-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1H-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% CO2 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% CO2 at 37°. Cell viability was assessed by trypan blue dye exclusion.

Measurement of [Ca2+]i. [Ca2+]i, was determined with the dye fura-2/AM, as described (10), with modifications. Cells were suspended at 1 x 10⁶ cells/ml in a buffer consisting of (in mM) 138 NaCl, 6 KCl, 1 MgSO₄, 1 NaH₂PO₄, 5 NaHCO₃, 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 x 10⁵ 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 x 10⁶ cells/ml and were centrifuged at 250 x g for 10 min at 20°. Cells were suspended at 1.0 x 10⁶ cells/ml in the aforementioned buffer and were kept at 20° until measurement of [Ca2+]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 [Ca2+]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 [Ca2+]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 x 10⁶ cells) were suspended in 2 ml of the aforementioned buffer, using acryl fluorescence cuvettes (Sarstedt, Numbrecht, Germany). Fluorescence was determined at 37°, with constant stirring of the cells at 1 x 10⁴ 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 extracellular substances (e.g., histamine receptor antagonists), before the addition of stimuli; basal fluorescence (basal [Ca2+]i,) was measured for 1 mm. The excitation and emission wavelengths were 340 and 500 nm, respectively. Basal [Ca2+]i values and peak [Ca2+]i values stimulated by various agonists were calculated according to eq. 6 given in Ref. 16. Basal [Ca2+]i values were subtracted from the corresponding peak [Ca2+]i values, to calculate the increase in [Ca2+]i, induced by a given agonist. Unless stated otherwise, all experiments were performed in the presence of extracellular Ca2+ (1 mM CaCl2 added to the buffer 3 min before stimuli). Quantitative comparison of peak [Ca2+]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 (in [Ca2+]i values in the challenged aliquot of cells had already declined), the fresh aliquot was placed into the fluorometer, to assess basal [Ca2+]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 μM myo-[2-3H]inositol, in a humidified atmosphere with 7% CO2 at 37°. Pretreated cells were centrifuged at 250 x g for 10 min at 20°. Cells were suspended in the buffer used for the determination of [Ca2+]i. Cells were re centrifuged and suspended in buffer. After another centrifugation of the cells, they were suspended at 1 x 10⁶ cells/ml in buffer. Reactions were performed at 37° in buffer supplemented with 1 mM CaCl2, 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 CHCl3, CH3OH, and concentrated HCl (100:200:1, v/v). Thereafter, 125 μl of CHCl3 and 25 μl of H2O were added to the reaction mixtures. After centrifugation for phase separation, 350 μl of the aqueous phase were loaded on Dowex 1 x 8 columns (0.8 x 2 cm). Inositol phosphates were eluted as described (17). The eluates (6 ml) were mixed with 12 ml of Flow-sint IV scintillation fluid (Camberra Packard, Frankfurt/Main, Germany), and radioactivity was determined by scintillation counting.

Calculations. EC50 and IC50 values were obtained by graphically analyzing the concentration-response curves shown in Figs. 1-4.

Results

Histamine and dimaprit induce increases in [Ca2+]i in HL-60 promyelocytes. Histamine increased [Ca2+]i, with an EC50 of 15 μM and a maximum at 0.3-1.0 μM (Fig. 1). Dimaprit increased [Ca2+]i, with an EC50 of 30 μM and a maximum at 100 μM. The effectiveness of dimaprit (1 mM) to increase [Ca2+]i amounted to 20% of that of histamine (1 mM). Pertussis toxin ADP-riboseylates heterotrimeric regulatory guanine nucleotide-binding proteins of the Gt 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 [Ca2+]i, (see Fig. 1).

Betalazine (a weak partial H₁ agonist) (6, 18), aripiprazole (a potent H₂ agonist and H₁ antagonist) (11), impromidine (a potent H₁ agonist and H₂ antagonist and weak H₂ antagonist) (6, 18), and (R)-α-methylhistamine (a potent H₂ agonist) (18, 19), at concentrations up to 100 μM, did not induce rises in [Ca2+]i; in HL-60 promyelocytes (data not shown). Diphenhydramine (an H₁ antagonist) (6, 18) and cimetidine and famotidine (H₂ antagonists) (18, 20), at up to 100 μM each, did not affect basal [Ca2+]i, or induce a rise in [Ca2+]i, in HL-60 promyelocytes (data not shown). Diphenhydramine, cimetidine, famotidine, and imipramine, at up to 100 μM each, showed no inhibitory effect on rises in [Ca2+]i, induced by ATP (10 μM) (data not shown). The cell-permeant cAMP analogue dibutyryl-cAMP (1 mM) did not induce rises in [Ca2+]i, (data not shown).

H₁ and H₂ antagonists and imipramine inhibit histamine-induced rises in [Ca2+]i, in HL-60 promyelocytes. The effects of diphenhydramine, cimetidine, famotidine, and imipramine on the rises in [Ca2+]i, induced by histamine (100 μM) were studied (Fig. 2). Diphenhydramine reversed stimulation caused by histamine with an IC50 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 IC50 values of 300 nM and 85 nM, respectively, and both substances at 100 μM diminished the histamine-induced rises in [Ca2+]i,
or its vehicle (control) for 24 hr. Thereafter, cells were harvested and loaded with fura-2/AM, and the increases in [Ca\textsuperscript{2+}] induced by histamine or dimaprit were assessed. Control cells stimulated with histamine; \( \bigcirc \), pertussis toxin-treated cells stimulated with histamine; \( \Delta \), control cells stimu-

lated with dimaprit; \( \Psi \), pertussis toxin-treated cells stimulated with dimaprit. Substances at the designated concentrations did not increase [Ca\textsuperscript{2+}]. Data shown are the means \pm standard deviations of four experiments performed with different preparations of HL-60 cells.

Impromidine antagonized the effects of histamine with an IC\textsubscript{50} of 100 nM, and inhibition was complete at 100 \( \mu \)M. Famotidine (100 \( \mu \)M) abolished the stimulatory effect of dimaprit (100 \( \mu \)M) on [Ca\textsuperscript{2+}], whereas diphenhydramine (100 \( \mu \)M) 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 \( \mu \)M each). Diphenhydramine reduced the effectiveness of histamine to increase [Ca\textsuperscript{2+}], without changing its EC\textsubscript{50}. Famotidine increased the EC\textsubscript{50} for histamine by about 4-fold and greatly reduced its effectiveness. Marked reductions in the effectiveness of histamine (1 \( \mu \)M to 3 mM) to induce rises in [Ca\textsuperscript{2+}] were also observed with famotidine at 100 nM, 1 \( \mu \)M, and 10 \( \mu \)M (data not shown). The combination of diphenhydramine plus famotidine (100 \( \mu \)M each) abolished the stimulatory effects of histamine, at concentrations as high as 3 mM, on [Ca\textsuperscript{2+}], (see Fig. 3).

The effects of impropidine 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\textsuperscript{2+}]. Impromidine at up to 1 \( \mu \)M did not substantially increase the EC\textsubscript{50} for histamine. Impromidine at 10 and 100 \( \mu \)M increased the EC\textsubscript{50} for histamine by about 6- and 30-fold, respectively. Cimetidine greatly depressed the concentration-response curve for histamine and slightly increased its EC\textsubscript{50}.

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

Histamine activates phosphoinositide degradation in HL-60 promyelocytes. A small part of the histamine-induced rise in [Ca\textsuperscript{2+}], in HL-60 promyelocytes was due to mobilization of Ca\textsuperscript{2+} from intracellular stores (see Fig. 5). Because Ca\textsuperscript{2+} mobilization is preceded by formation of InsP\textsubscript{3} (7), the effects of histamine on phosphoinositide degradation were studied. In agreement with the small effect of histamine on Ca\textsuperscript{2+} mobilization, histamine slightly stimulated formation of InsP\textsubscript{3} (Table 1).

Effects of ATP and histamine on [Ca\textsuperscript{2+}], in various human myeloid cell types. ATP induces increases in [Ca\textsuperscript{2+}], 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\textsuperscript{2+}], in various
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\(^{2+}\)] in HL-60 promyelocytes (Table 2; see also Table 1). ATP also effectively increased [Ca\(^{2+}\)] in U-937 promonocytes and in HEL cells, whereas in K-562 cells ATP did not induce rises in [Ca\(^{2+}\)]. Unlike ATP, histamine did not show stimulatory effects on [Ca\(^{2+}\)] in U-937 promonocytes and in HEL cells, and it also did not induce rises in [Ca\(^{2+}\)] 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\(^{2+}\)]. 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\(^{2+}\)] 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). Similarly, 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\(^{2+}\)] 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\(^{2+}\)] in HL-60 promyelocytes were due to histamine-induced rises in cAMP, inasmuch as a cell-permeant analogue of cAMP did not increase [Ca\(^{2+}\)]. Surprisingly, the effects of histamine on [Ca\(^{2+}\)] in HL-60 promyelocytes are mediated largely via H\(_2\) receptors and not via H\(_3\) 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
by histamine (100 μM) were assessed. Arrow, addition of histamine. Three minutes before histamine, various substances were added to cells. Trace 1, CaCl₂ (1 mM); trace 2, CaCl₂ (1 mM) plus SK&F 96365 (10 μM); trace 3, EGTA (1 mM) without added CaCl₂. 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

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Inositol phosphates*</th>
<th>dpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent (control)</td>
<td>1042 ± 179</td>
<td>948 ± 49</td>
</tr>
<tr>
<td>Histamine</td>
<td>885 ± 52</td>
<td>925 ± 129</td>
</tr>
<tr>
<td>ATP</td>
<td>1353 ± 36</td>
<td>3021 ± 198</td>
</tr>
</tbody>
</table>

*InsP₁, inositol monophosphate; InsP₂, inositol bisphosphate.

**TABLE 2**

Effects of ATP and histamine on [Ca²⁺] in various human myeloid cell types

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Increase in [Ca²⁺]</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT-1082 promyelocytes</td>
<td>1084 ± 103</td>
</tr>
<tr>
<td>K-562 cells</td>
<td>360 ± 57</td>
</tr>
<tr>
<td>U-937 promonocytes</td>
<td>664 ± 134</td>
</tr>
<tr>
<td>HEL cells</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

**TABLE 3**

Effects of histamine, diphenhydramine, and famotidine on fMet-Leu-Phe-induced rises in [Ca²⁺] in HL-60 cells

<table>
<thead>
<tr>
<th>Addition</th>
<th>HL-60 promyelocytes</th>
<th>HL-60 cells (histamine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent (control)</td>
<td>105 ± 8</td>
<td>447 ± 26</td>
</tr>
<tr>
<td>Diphenhydramine (10 μM)</td>
<td>109 ± 12</td>
<td>468 ± 34</td>
</tr>
<tr>
<td>Famotidine (10 μM)</td>
<td>98 ± 10</td>
<td>110 ± 12</td>
</tr>
</tbody>
</table>

H₂ agonist (dimaprit) increased [Ca²⁺]; in HL-60 promyelocytes to some extent (see Fig. 1), whereas a full H₂ agonist and a partial H₂ 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 [Ca²⁺], in HL-60 promyelocytes is also in accordance with the aforementioned assumption, because H₂ receptors couple to the pertussis toxin-insensitive guanine nucleotide-binding protein G, (see Fig. 1) (6, 18, 25). Whether H₂ receptors additionally couple to one or more of the recently cloned pertussis toxin-insensitive guanine nucleotide-binding proteins, e.g., G₁₁ or G₁₂, to activate phospholipase C and/or nonselective cation channels, remains to be determined (26). Interestingly, in dimethyl sulfoxide-differentiated HL-60 cells, H₂ receptors mediate increases in [Ca²⁺], via pertussis toxin-insensitive guanine nucleotide-binding proteins as well (27). Moreover, H₂ receptor-mediated rises in [Ca²⁺], were reported for gastric parietal cells (28). Thus, stimulation of rises in [Ca²⁺] via H₂ receptors is not restricted to HL-60 promyelocytes but may be a more general phenomenon.

**Does a H₂ receptor subtype mediate the histamine-induced rises in [Ca²⁺] in HL-60 promyelocytes?** The E₉₀ values for various H₂ 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 H₂ receptor-mediated increases in [Ca²⁺] 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 guinea pig atrium (standard model for the characterization of H₂ receptors) (1–3, 6, 11, 14, 18, 20). Impromidine, in addition to being an H₂ agonist, is a potent H₂ and weak H₁ antagonist (6, 18, 19). Interestingly, impromidine is a partial agonist at H₂ receptors in various systems, including H₂ 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 [Ca²⁺] in HL-60 promyelocytes (1, 3, 11, 29). Instead, impromidine reversed histamine-induced rises in [Ca²⁺], 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 [Ca2+], induced by histamine at 100 μM (see Figs. 2 and 4). It is unlikely that histamine acted via H3 receptors, because (R)-α-methylhistamine, a potent and selective H3 agonist (6, 18, 19), did not increase [Ca2+]. Additionally, dimaprit, which shows H2 antagonistic effects (19), increased [Ca2+] in HL-60 promyelocytes (see Fig. 1). Moreover, the stimulatory effects of dimaprit on [Ca2+] were inhibited by famotidine. Thus, it appears that imiprodine acted as a dual H2/H3 antagonist to inhibit histamine-induced rises in [Ca2+], in HL-60 promyelocytes (see also below).

Similarly to imiprodine, the structurally related guanidine apromidin (11) failed to induce rises in [Ca2+], 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, apromidine 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 H2 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 apromidine-induced inhibition of superoxide formation (1–3, 14, 29). With respect to histamine-induced increases in [Ca2+], in HL-60 promyelocytes, famotidine, cimetidine, and imiprodine showed marked noncompetitive antagonism (see Figs. 3 and 4). It is, however, unlikely that these substances inhibited rises in [Ca2+] in HL-60 promyelocytes in a nonspecific manner, because they did not inhibit ATP-induced rises in [Ca2+]. 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 [Ca2+], 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 H2 receptor-mediated increases in cAMP and in [Ca2+] in HL-60 promyelocytes are quite different. Additionally, there are substantial pharmacological differences between H2 receptor-mediated increases in [Ca2+] in HL-60 promyelocytes and responses in other systems. These data suggest that histamine mediates increases in cAMP and in [Ca2+] in HL-60 promyelocytes through different receptor subtypes.

What may be the function of H2 receptor-mediated increases in [Ca2+] 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 H2 receptors, inasmuch as it was abolished by famotidine (see Table 3). These data support the notion that H2 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 H2 antagonists may lead to neutropenia or agranulocytosis (1, 2, 35).

In HL-60 promyelocytes, histamine induces parallel increases in cAMP and in [Ca2+], (see Figs. 1–5) (1). Ca2+ influx through nonselective cation channels is required for histamine-induced differentiation, inasmuch as blockade of these channels by SK&F 96385 was cytotoxic and prevented differentiation. H1 receptor-mediated rises in [Ca2+] may potentiate H2 receptor-mediated cAMP accumulation (6). Similarly, H2 receptor-mediated rises in [Ca2+] in HL-60 promyelocytes may potentiate H2 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 [Ca2+], 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 E2 is another inducer of differentiation of HL-60 cells (36). The effectiveness of prostaglandin E2 to induce rises in cAMP is greater in HL-60 promyelocytes than in human neutrophils, and prostaglandin E2 induces rises in [Ca2+] in the former cells but not in the latter (1, 29, 15, 37). Thus, histamine- and prostaglandin E2-induced rises in [Ca2+] in HL-60 promyelocytes may amplify cAMP accumulation, resulting in the induction of differentiation.

Not only H2 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 [Ca2+] 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 Ca2+ ionophore A23187 induces differentiation of HL-60 promyelocytes as well (38, 39). Thus, an increase in [Ca2+], may provide a sufficient signal for the induction of differentiation, independently of cAMP accumulation. H2 receptor-mediated rises in [Ca2+] do not play a role in the induction of differentiation of U-937, HEL, and K-562 cells, inasmuch as histamine did not increase [Ca2+] in these cells (see Table 2). In contrast, ATP increased [Ca2+] in U-937 promonocytes and in HEL cells. Additionally, ATP was substantially more effective than histamine in activating phosphoinositide degradation and inducing rises in [Ca2+] in HL-60 promyelocytes (see Tables 1 and 2) (7, 10, 15). These data suggest that ATP and H2 receptor-mediated rises in [Ca2+], play different roles in the regulation of myeloid differentiation processes.

H2 receptors mediate a small portion of histamine-induced rises in [Ca2+], in HL-60 promyelocytes. Evidence in support of the notion that H2 receptors mediated a small part of the histamine-induced rises in [Ca2+], 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 [Ca2+] (see Fig. 3). With histamine at 100 μM, the H2 antagonist-insensitive portion of rises in [Ca2+] amounted to about 25%, presumably reflecting the H2 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 H2 antagonistic properties of diphenhydramine at the high concentration used (see Fig. 2) (1, 29). The IC50 for diphenhydramine on rises in [Ca2+] 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 imiprodine support the view that H2 receptors mediated, to a small extent, histamine-induced rises...

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