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Receptor-Independent G Protein Activation May Account for the Stimulatory Effects of First-Generation H_1-Receptor Antagonists in HL-60 Cells, Basophils, and Mast Cells

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ABSTRACT. The first-generation histamine H_1-receptor antagonists, chlorpheniramine (CPHE) and diphenhydramine (DPH), may activate histamine release from basophils and mast cells. Because CPHE and DPH are cationic-amphiphilic and because several substances with such physicochemical properties activate heterotrimeric regulatory guanine nucleotide-binding proteins (G-proteins) in a receptor-independent manner, we asked the question of whether or not H_1-receptor antagonists could be G-protein activators as well. In dibutyryl cAMP-differentiated HL-60 cells, CPHE and DPH increased cytosolic Ca^{2+} concentration and azurophilic granule release in pertussis toxin (PTX)-sensitive manners. In HL-60 membranes, PTX-sensitive stimulations of GTPase [E.C. 3.6.1.-] and binding of guanosine 5′-[γ-thio]triphosphate by H_1 receptor antagonists were observed. CPHE and DPH also increased GTP hydrolysis by the purified PTX-sensitive G-protein, transducin. In all-*trans*-retinoic acid-differentiated HL-60 cells and rat basophilic leukemia cells (RBL 2H3 cells), H_1-receptor antagonists induced, unlike in dibutyryl cAMP-differentiated HL-60 cells, Ca^{2+} influx without Ca^{2+} mobilization from intracellular stores. CPHE and DPH also induced serotonin release from RBL 2H3 cells. Our data indicate that first-generation H_1-receptor antagonists are receptor-independent G-protein activators and that such a mechanism of action accounts for their stimulatory effects in HL-60 cells, basophils, and mast cells. BIOCHEM PHARMACOL 51;2:125–131, 1996.

KEY WORDS. Ca^{2+} influx; G-proteins; H_1-receptor antagonists; HL-60 leukemic cells; pertussis toxin; rat basophilic leukemia (RBL 2H3) cells

Histamine H_1-receptor antagonists are widely used in the treatment of allergic disorders, e.g., rhinoconjunctivitis, urticaria and anaphylaxis [1, 2]. However, at concentrations between 0.1–10 mM, first-generation H_1-receptor antagonists such as DPH,‡ CPHE and PRO induce histamine release from basophils and mast cells [3–6]. The mechanism underlying these effects of H_1-receptor antagonists is poorly understood. A property that first-generation H_1-receptor antagonists have in common with the classical mast cell-activating substances, substance P and compound 48/80, is their cationic-amphiphilic character [1, 2, 7]. It has been shown that compound 48/80 and substance P activate purified pertussis toxin (PTX)-sensitive heterotrimeric regulatory G-proteins in a receptor-independent manner and that such a mechanism presumably accounts for their stimulatory effects in mast cells [7, 8]. Interestingly, various cationic-amphiphilic ligands at histamine receptors (i.e. 2-substituted histamines, lipophilic arpromidine-derived guanidines, and a histamine trifluoromethyl-toluidide derivative) are receptor-independent G-protein activators, as well [9–11].

On the basis of the aforementioned findings, we asked the question of whether or not receptor-independent G-protein activation could explain the pro-inflammatory effects of first-generation H_1-receptor antagonists. As model systems for our study, we employed the human leukemia cell line, HL-60, the rat basophilic leukemia cell line, RBL 2H3, and a purified PTX-sensitive G-protein, TD. HL-60 cells have already been proven to be a useful model system for the analysis of receptor-independent G-protein activation [9–12]. These cells express similar G-proteins and effector systems as basophils and mast cells and, unlike the latter, can be obtained in virtually unlimited quantities [12–14]. In addition, the nucleotidase activity in HL-60 membranes, unlike in rat peritoneal mast cells, is low, a property that greatly facilitates studies on G-protein activation [15, 16]. Moreover, by analogy to mast cells from

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‡ *Abbreviations:* Bt_2cAMP, dibutyryl cAMP; DNP, dinitrophenyl; $[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; CPHE, chlorpheniramine; DPH, diphenhydramine; G-protein, heterotrimeric regulatory guanine nucleotide-binding protein; GTPγS, guanosine 5′-[γ-thio]triphosphate; PRO, promethazine; PTX, pertussis toxin; RA, all-*trans*-retinoic acid; TD, transducin.

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different tissues [17], there is substantial variability in the responsiveness towards receptor-independent G-protein activators of HL-60 cells that had been subjected to different differentiations [10]. With respect to RBL 2H3 cells, little is known about receptor-independent G-protein activation because they do not respond to compound 48/80 and substance P in the native state [18]. Purified TD is activated by mastoparan, arpromidine-derived guanidines, and a histamine trifluoromethyl-toluidide derivative [11, 19].

MATERIALS AND METHODS

Materials

CPHE, DPH, and PRO were obtained from Sigma Chemie (Deisenhofen, Germany). Stock solutions of CPHE and DPH (0.1 M) and PRO (10 mM) were prepared in distilled water and stored at −20°. Sources of other materials have been described elsewhere [9–12, 16, 20–25].

Cell Culture and Membrane Preparation

HL-60 cells were cultured in suspension and differentiated towards neutrophil-like cells upon incubation with dibutyryl cAMP (Bt_2cAMP) (0.2 mM) for 48 hr [16] or with RA (10 nM) for 168 hr [20]. Twenty four hr before experiments or membrane preparation, PTX (100 ng/mL) or its carrier (control) was added to cell cultures. Culture of RBL 2H3 cells and sensitization with DNP-IgE were performed as described [21]. HL-60 membranes were prepared according to Seifert and Schultz [22].

Purification of TD

Illuminated rod outer segment disk membranes were prepared as described by Papermaster and Dreyer [26]. TD was eluted from membranes with 100 μM GTP according to Kroll *et al.* [27]. GTP was removed from TD by repeated concentration and dilution with GTP-free buffer in an Amicon concentration chamber (PM 10 membrane). The purity of TD was >98% as assessed by SDS-PAGE [23] and silver staining [20]. Stock solutions of TD (4–5 μM) were stored at −70°.

Measurement of Cytosolic Ca^{2+} Concentration ($[Ca^{2+}]_i$) in RBL 2H3 Cells

Cells (3×10^4 cells) were seeded in cell culture dishes (diameter 3.5 cm) in which a glass coverslip (2 cm × 2 cm × 0.07 mm) had been placed, and were cultured for 12 hr as described [21]. Therefore, the culture medium was decanted and cells were loaded with fura-2/acetoxymethylester (2 μM) for 30 min at 37° in a buffer consisting of (mM) 138 NaCl, 6 KCl, 0.1 $CaCl_2$, 1 $MgSO_4$, 1 Na_2HPO_4, 5 $NaHCO_3$, 5.5 glucose, and 20 HEPES/NaOH, pH 7.4, substituted with 0.1% (w/v) bovine serum albumin. Extracellular dye was removed by washing the cells twice with 2 mL of a buffer consisting of (mM) 138 NaCl, 6 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5.5 glucose and 20 Hepes/NaOH, pH 7.4. For measurement of $[Ca^{2+}]_i$, cells were overlayered with 1 mL of the aforementioned buffer supplemented with 1 mM $CaCl_2$ or 1 mM EGTA. Determination of $[Ca^{2+}]_i$ in single cells was performed at 25° using an imaging system (T.I.L.L., Photonics, Munich, Germany). Cells were visualized using an inverted microscope (Zeiss axiovert 1000) and Neor fluor 40× oil immersion objective (Zeiss, Oberkochen, Germany). Fura-2 fluorescence was excited alternately at 340 nm and 380 nm *via* microscope port with illumination provided by a 100 W mercury lamp (Olympus, Tokyo, Japan). Cellular fluorescence was filtered through a 510 nm band pass filter. F_{max} and F_{min} were determined by subsequent addition of ionomycin (10 μM) and EGTA (10 mM), respectively, to cells in medium containing $CaCl_2$ (1 mM). Images were digitized onto a Pentium 66 Mhz computer and analyzed by the Fucal 5.00 soft ware (T.I.L.L., Photonics, Munich, Germany). Ratiometric Ca^{2+} images were generated at 3 sec intervals. For background compensation, illumination of an area containing no cells was subtracted. For each cell, $[Ca^{2+}]_i$ was averaged from pixels within manually outlined cell areas.

Other Assays with Intact Cells

$[Ca^{2+}]_i$ in HL-60 cells was determined according to Seifert *et al.* [24], using the fluorescent dye, fura-2. The release of β-glucuronidase [EC 3.2.1.31] and lactate dehydrogenase [EC 1.1.1.27] from HL-60 cells was determined as described [25, 28]. Measurement of serotonin release from RBL 2H3 cells was performed according to Offermanns *et al.* [21]. Reactions were conducted for 30 min.

Assays with HL-60 Membranes and TD

GTP hydrolysis and [^{35}S]guanosine 5′-[γ-thio]triphosphate (GTPγS) binding in HL-60 membranes were determined according to Seifert *et al.* [9]. GTP hydrolysis by TD (160 nM) was performed as for HL-60 membranes, except that the incubation time was 30 min.

Miscellaneous

Protein was determined according to Lowry *et al.* [29]. [γ-^{32}P]GTP was synthesized according to Walseth *et al.* [30].

RESULTS

An early event in the activation process of HL-60 cells is mobilization of Ca^{2+} from intracellular stores and stimulation of Ca^{2+} influx from the extracellular space [9, 24]. In the presence of extracellular Ca^{2+}, CPHE (1 mM) induced a rapid increase in $[Ca^{2+}]_i$ which, after an initial peak, returned to a plateau above basal values (Fig. 1). In the absence of extracellular Ca^{2+}, the effect of CPHE (1 mM) was smaller and more transient. These data indicate that CPHE activated Ca^{2+} mobilization from intracellular stores and sustained Ca^{2+} influx from the extracellular space. Similar data as with CPHE were obtained with DPH (data not shown). FMLP was more effective than H_1-receptor antagonists at activating Ca^{2+} mo-

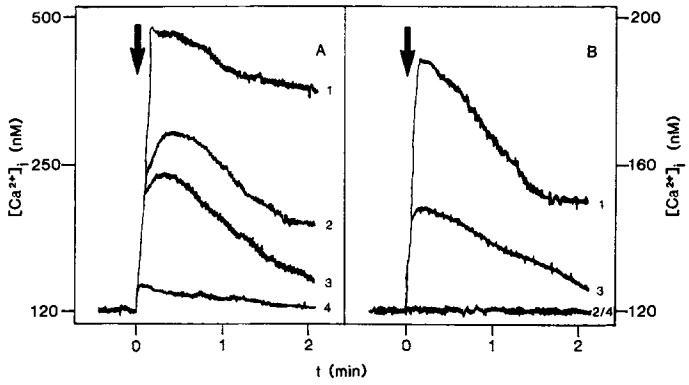

FIG. 1. Time courses of FMLP- and CPHE-induced rises in $[Ca^{2+}]_i$ in suspended Bt_2cAMP-differentiated HL-60 cells: effects of extracellular Ca^{2+} and PTX. Treatments of HL-60 cells with PTX (traces 2 and 4) or carrier (control) (traces 1 and 3) were performed as described in "Materials and Methods." Cells were harvested, loaded with fura-2/acetoxymethylester as described in "Materials and Methods," and the effects of FMLP (1 μM) (A) or CPHE (1 mM) (B) on $[Ca^{2+}]_i$ were assessed in the presence of 1 mM extracellular $CaCl_2$ (traces 1 and 2) or 1 mM EGTA (traces 3 and 4). Arrows indicate the addition of stimulus to cells. Superimposed original fluorescence tracings obtained in a single experiment are shown. Similar results were obtained in at least 3 independent experiments performed with different cell preparations.

bilization and Ca^{2+} influx. PTX ADP-ribosylates G_i-protein α-subunits and, thereby, inhibits their interaction with agonist-occupied formyl peptide receptors and cationic-amphiphilic substances [9]. The stimulatory effects of CPHE and DPH on $[Ca^{2+}]_i$ were fully PTX-sensitive, whereas the effect of FMLP, in particular in the presence of extracellular Ca^{2+}, was only partially PTX-sensitive (see Fig. 1) [24].

A late event in the signal transduction cascade in HL-60 cells is stimulation of azurophilic granule release. CPHE and DPH (1 mM each) stimulated β-glucuronidase release by approximately 80% and 65% above basal values, respectively (Table 1). FMLP increased azurophilic granule release by more than three-fold above basal. The stimulatory effects of FMLP, CPHE, and DPH on β-glucuronidase release were PTX-sensitive.

TABLE 1. Stimulation of β-glucuronidase release by FMLP, CPHE, and DPH in Bt_2-cAMP-differentiated HL-60 cells: effect of PTX

	β-Glucuronidase release (% of cellular content)	
Stimulus	**Carrier (control)**	**PTX**
None (basal)	9.0 ± 0.2	9.7 ± 0.5
FMLP (1 μM)	31.8 ± 1.5*	9.8 ± 0.1†
CPHE (1 mM)	16.3 ± 0.4*	11.1 ± 0.5†
DPH (1 mM)	14.9 ± 0.4*	10.5 ± 0.8†

Treatment with PTX and measurement of azurophilic granule release were performed as described in Materials and Methods. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in at least 3 independent experiments peformed with different cell preparations. The statistical significance of the effects of stimuli compared to basal azurophilic granule release was assessed using the Wilcoxon test.

*$P < 0.05$.

†Not significant.

To answer the question of whether or not H_1-receptor antagonists induce cell lysis, we assessed the release of a cytosolic enzyme, lactate dehydrogenase. Basal lactate dehydrogenase release was 8.5 ± 1.3% of the cellular content (mean ± SD, $n = 4$), and CPHE and DPH (1 mM each) reduced this enzyme release to 4.4 ± 0.5% and 3.0 ± 0.6%, respectively.

Next, we studied the effects of FMLP and H_1-receptor antagonists on G-protein activation in HL-60 membranes. A physiological parameter in this regard is measurement of high-affinity GTP hydrolysis catalyzed by G-protein α-subunits [11, 14]. CPHE increased GTP hydrolysis with a half-maximal effect at 0.4 mM and a plateau at 1–3 mM (Fig. 2). In accordance with the data concerning intact cells, FMLP was a more effective activator of GTP hydrolysis than CPHE and DPH (Table 2). Another H_1-receptor antagonist known to induce activation of basophils and mast cells, PRO, was also an activator of GTPase [EC 3.6.1.-]. In membranes of PTX-treated cells, the stimulatory effects of FMLP and H_1-receptor antagonists on GTP hydrolysis were abolished. We noted that the H_1-receptor antagonists even inhibited GTP hydrolysis in membranes of PTX-treated cells by 24–33%, but do not have a mechanistic explanation for this finding. GTPγS is a hydrolysis-resistant GTP analogue that binds to G-protein α-subunits with high affinity [11, 14]. CPHE activated GTPγS binding in HL-60 membranes with a half-maximal effect at 0.1 mM and a maximum at 1 mM (see Fig. 2). The effect of CPH on GTPγS binding was PTX-sensitive (data not shown).

In subsequent experiments, we studied the effects of CPHE and DPH on GTP hydrolysis by purified TD. CPHE activated the G-protein with a half-maximal effect at 1.5 mM and a maximum at 5 mM (Fig. 3). At a maximally effective concen-

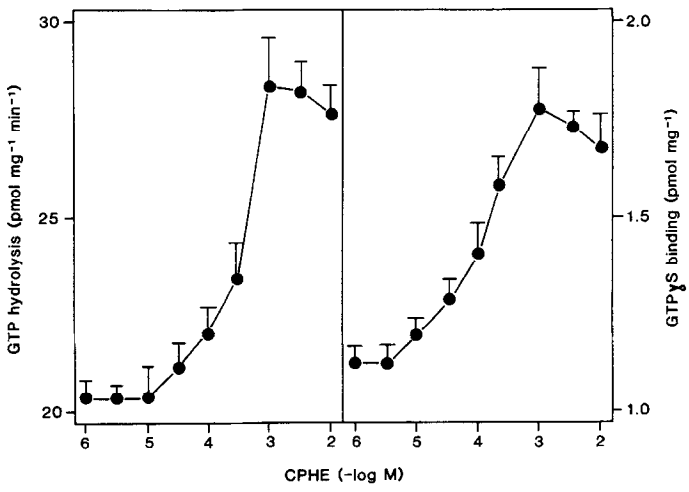

FIG. 2. Concentration-response curves for the stimulatory effects of CPHE on GTP hydrolysis and GTPγS binding in HL-60 membranes. GTP hydrolysis and GTPγS binding were determined in the presence of CPHE at the indicated concentrations as described in Materials and Methods. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained with 3 different membrane preparations.

TABLE 2. Stimulation of GTP hydrolysis by FMLP, CPHE, DPH, and PRO in membranes of Bt_2-cAMP-differentiated HL-60 cells: effect of PTX

Stimulus	GTP hydrolysis (pmol $mg^{-1}min^{-1}$)	
	Carrier (control)	PTX
None (basal)	18.5 ± 0.7	11.1 ± 0.4
FMLP (10 μM)	46.6 ± 1.5*	12.0 ± 0.6†
CPHE (1 mM)	25.7 ± 1.0*	7.4 ± 0.4*
DPH (1 mM)	27.0 ± 0.9*	8.8 ± 0.5*
PRO (0.1 mM)	24.4 ± 0.8*	7.4 ± 0.3*

Treatment with PTX and measurement of GTP hydrolysis were performed as described in Materials and Methods. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained with 3 different membrane preparations. The statistical significance of the effects of stimuli compared to basal GTP hydrolysis was assessed using the Wilcoxon test.

*$P < 0.05$.

†Not significant.

tration, CPHE increased enzyme activity by more than three-fold above basal. DPH was also an activator of GTP hydrolysis catalyzed by TD. Its effect was half-maximal at 0.75 mM, and a maximum was reached at 5 mM. At this concentration, DPH increased GTP hydrolysis by approximately 2.5-fold above basal.

It is well-known that certain receptor-independent G-protein activators exhibit substantial cell type specificity in their effects [10, 17, 18]. To address this issue, we studied the effects of H_1-receptor antagonists in RA differentiated HL-60 cells and RBL 2H3 cells. In the former, formyl peptide receptors are partially uncoupled from G_i-proteins [31]. This alteration of receptor/G-protein interaction is reflected by the finding that, in RA-differentiated HL-60 cells, FMLP-induced rises in $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+} declined more rapidly than in Bt_2cAMP-differentiated HL-60 cells (compare Figs. 1 and 4). Similar findings were obtained for CPH-induced rises in $[Ca^{2+}]_i$. Interestingly, in RA-differentiated HL-60 cells, CPHE did not induce rises in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+} (i.e. the substance activated Ca^{2+} influx without concomitant Ca^{2+} mobilization). CPHE-induced Ca^{2+} influx in RA-differentiated HL-60 cells was completely PTX-sensitive, whereas the effect of FMLP was not fully inhibited by toxin treatment.

Cross-linking of high-affinity IgE receptors in RBL 2H3 cells resulted in a large and sustained rise in $[Ca^{2+}]_i$ (Fig. 5) that was fully attributable to Ca^{2+} influx from the extracellular space (Table 3). Lin and Gilfillan [32] obtained results similar to those reported here. DPH (1 mM) increased $[Ca^{2+}]_i$ in RBL 2H3 cells by stimulating Ca^{2+} influx, but the peak $[Ca^{2+}]_i$ values were lower than with antigen stimulation (see Fig. 5 and Table 3). Compared to DPH, CPHE (1 mM) induced a much more transient Ca^{2+} influx although the peak $[Ca^{2+}]_i$ values stimulated by both H_1-receptor antagonists were of similar magnitude. Unlike antigens, CPHE and DPH, the adenosine receptor agonist 5′-N-ethylcarboxamido-adenosine increased $[Ca^{2+}]_i$ not only in the presence of extracellular Ca^{2+} but also to some extent in its absence, indicative for both activation of Ca^{2+} mobilization and Ca^{2+} influx (see Table 3).

Finally, we asked the question of whether or not antigen-, CPHE-, and DPH-induced Ca^{2+} influxes in RBL 2H3 cells possess a functional correlate. Therefore, serotonin release was

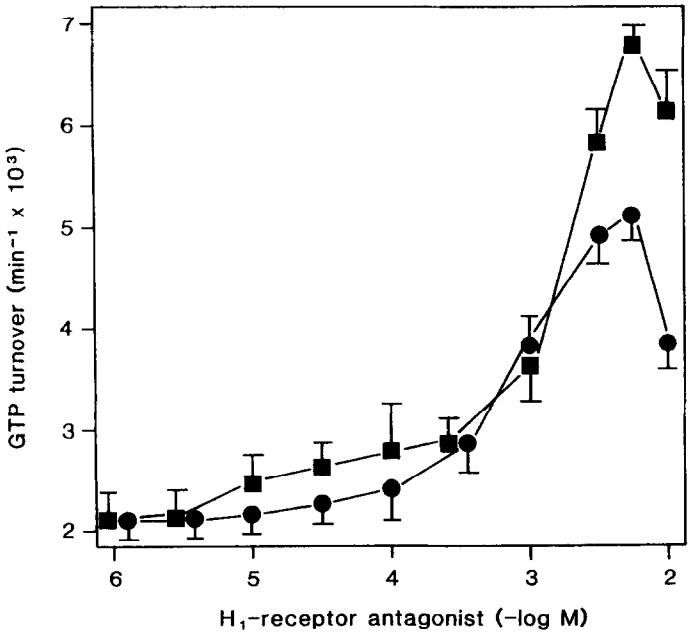

FIG. 3. Concentration-response curves for the stimulatory effects of CPHE and DPH on GTP hydrolysis catalyzed by purified TD. GTP hydrolysis was determined in the presence of CPHE (■) or DPH (●) at the indicated concentrations as described in "Materials and Methods." Data shown are the means ± SD of assay quadruplicates. Similar results were obtained with 2 different TD preparations.

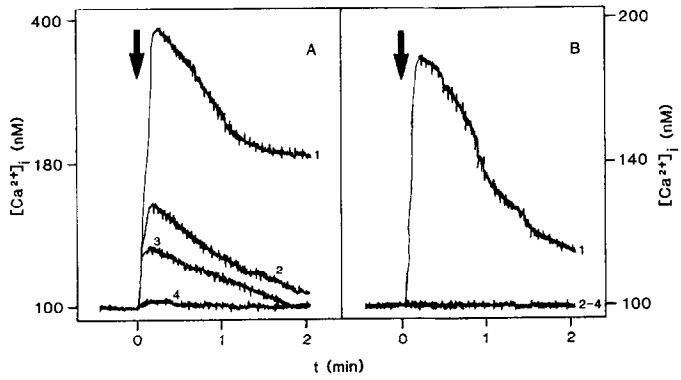

FIG. 4. Time-courses of FMLP- and CPHE-induced rises in $[Ca^{2+}]_i$ in suspended RA-differentiated HL-60 cells: effects of extracellular Ca^{2+} and PTX. Treatments of HL-60 cells with PTX (traces 2 and 4) or carrier (control) (traces 1 and 3) were performed as described in "Materials and Methods." Cells were harvested, loaded with fura-2/acetoxymethylester as described in "Materials and Methods," and the effects of FMLP (1 μM) (A) or CPHE (1 mM) (B) on $[Ca^{2+}]_i$ were assessed in the presence of 1 mM extracellular $CaCl_2$ (traces 1 and 2) or 1 mM EGTA (traces 3 and 4). Arrows indicate the addition of stimulus to cells. Superimposed original fluorescence tracings obtained in a single experiment are shown. Similar results were obtained in at least 3 independent experiments performed with different cell preparations.

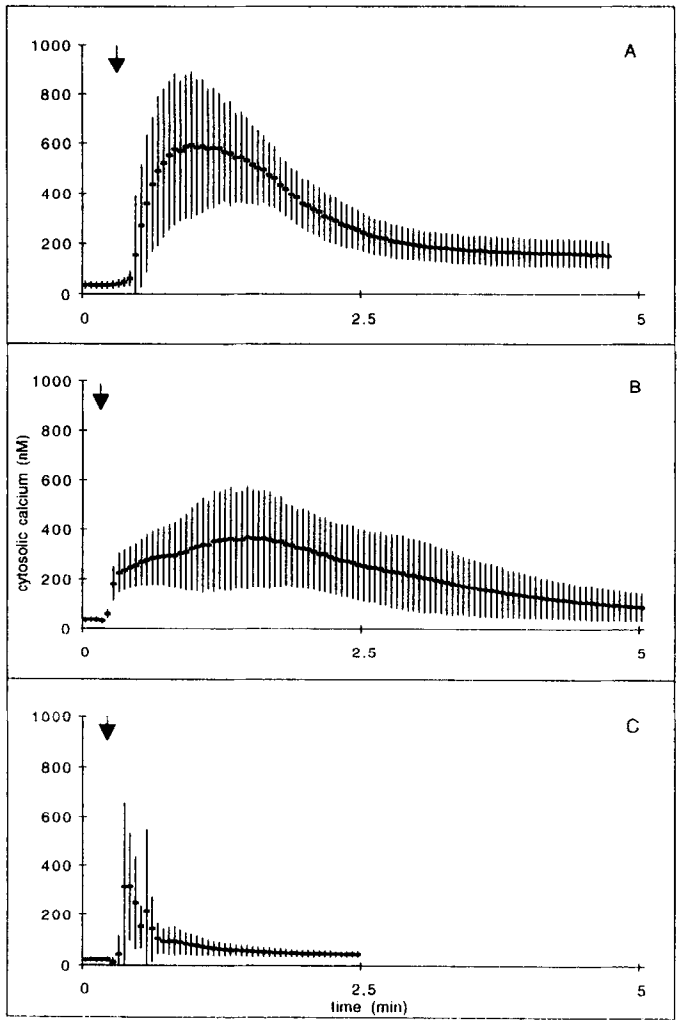

FIG. 5. Time courses of antigen-, DPH-, and CPHE-induced rises in $[Ca^{2+}]_i$ in adherent RBL 2H3 cells. Loading of cells with fura-2/acetoxymethylester and measurements of $[Ca^{2+}]_i$ were performed as described in "Materials and Methods." $[Ca^{2+}]_i$ was determined in the presence of 1 mM extracellular $CaCl_2$. (A) 2,4-DNP-albumin (100 ng/mL); (B) DPH (1 mM); and (C) CPHE (1 mM). Arrows indicate the addition of stimulus to cells. Bars indicate the SD values of 20 individual cells. Similar results were obtained with 3 different preparations of cells.

determined. 2,4-DNP-albumin (100 ng/mL) stimulated the release of 48.4 ± 7.3% of the cellular content of serotonin (mean ± SD, $n = 4$). The corresponding values for CPHE and DPH (1 mM each) were 18.9 ± 2.5% and 28.9 ± 1.4%, respectively.

DISCUSSION

First-generation H_1-receptor antagonists induce histamine release from basophils and mast cells [3–6]. In our present study, we show that these drugs also induce exocytosis in HL-60 leukemic cells and RBL 2H3 cells. It has been suggested that the H_1-receptor antagonist-induced release reactions are the result of cell lysis [6] (i.e. a nonspecific process). However, several findings argue against this notion. If H_1-receptor antagonists had acted in a toxic manner, they would have been expected not only to induce the release of granule content but also of cytosolic constituents [28, 33] (e.g. lactate dehydrogenase and fura-2). In contrast to this expectation, CPHE and DPH stabilized HL-60 membranes as is supported by the reduction in lactate dehydrogenase release. In addition, the increases in fura-2 fluorescence in HL-60 and RBL 2H3 cells induced by the drugs did not steadily increase but, rather, decreased with time, indicating that dye leakage into the extracellular space did not take place (see Figs. 1, 4, and 5) [33]. Moreover, CPHE was less effective than FMLP at increasing fura-2 fluorescence (see Figs. 1 and 4). Interestingly, Lichtenstein and Gillespie [5] also did not obtain convincing evidence for cytotoxic effects of H_1-receptor antagonists in basophils.

A clue to understanding the mechanism of action of H_1-receptor antagonists is the fact that PTX inhibited the stimulatory effects of CPHE and DPH on fura-2 fluorescence and azurophilic granule release in HL-60 cells (see Figs. 1 and 4 and Table 1). The PTX-sensitivity of the effects of H_1-receptor antagonists in intact HL-60 cells points to the involvement of G_i-proteins in the signal transduction pathway. In agreement with such a notion, the drugs induced PTX-sensitive stimulations of GTP hydrolysis and GTPγS binding in HL-60 membranes (see Table 2 and Fig. 2). These data suggest that first-generation H_1-receptor antagonists, by analogy to other cationic-amphiphilic ligands at histamine receptors [9–11], activate G_i-proteins in HL-60 cells in a receptor-independent manner.

To corroborate the assumption that H_1-receptor antagonists are receptor-independent G-protein activators, we studied their effects on the purified PTX-sensitive G-protein, TD. In accordance with the data concerning intact cells and cell membranes, first-generation H_1-receptor antagonists effectively stimulated GTP hydrolysis by TD (see Fig. 3). It should be emphasized that this G-protein activation took place in the absence of any phospholipids (i.e. intact membrane structures are not required for G-protein activation by H_1-receptor antagonists). These findings also argue against the hypothesis that H_1-receptor antagonists exert their stimulatory effects in basophils and mast cells by nonspecifically altering the physical properties of plasma membranes [6].

The mechanisms involved in the regulation of Ca^{2+} influx

TABLE 3. Antigen-, DPH-, CPHE-, and adenosine-induced rises in $[Ca^{2+}]_i$ in RBL 2H3 cells: effect of extracellular Ca^{2+}

	Increase in $[Ca^{2+}]_i$ (nM)	
Stimulus	**+$CaCl_2$**	**+EGTA**
2,4-DNP-albumin (100 ng/mL)	535 ± 294	0
NECA (10 μM)	123 ± 60	60 ± 40
CPHE (1 mM)	234 ± 98	0
DPH (1 mM)	295 ± 217	0

Loading of cells with fura-2/acetoxymethylester and measurements of $[Ca^{2+}]_i$ were performed as described in Materials and Methods. Peak $[Ca^{2+}]_i$ values induced by various stimuli were determined in the presence of 1 mM extracellular $CaCl_2$ or 1 mM extracellular EGTA. Basal $[Ca^{2+}]_i$ in RBL 2H3 cells was 70 ± 15 nM. Data shown are the means ± SD of 20 individual cells. Similar results were obtained with 3 different preparations of cells. NECA, N-ethylcarboxamidoadenosine.

in neutrophils/HL-60 cells and mast cells/RBL 2H3 cells are very complex. A well-known mechanism involves emptying of intracellular Ca^{2+} stores following phospholipase C activation, whereas other mechanisms are independent of Ca^{2+} store depletion [34–37]. In RA-differentiated HL-60 cells and RBL 2H3 cells, the two receptor-independent G-protein activators, CPHE and DPH, stimulated Ca^{2+} influx without concomitant Ca^{2+} mobilization (see Figs. 4 and 5 and Table 3). These data support the notion that certain Ca^{2+} influx pathways in human myeloid cells underlie a direct G-protein regulation [35, 36].

RBL 2H3 cells are nonresponsive towards compound 48/80 and substance P unless they are co-cultured with 3T3 fibroblasts for prolonged periods of time [18]. A lack of G-proteins in native RBL 2H3 cells cannot be the basis for this nonresponsiveness [38]. In accordance with the presence of G-proteins in native RBL 2H3 cells [38], first-generation H_1-receptor antagonists activated Ca^{2+} influx (see Fig. 5 and Table 3) and serotonin release. Whereas, in HL-60 cells, CPHE and DPH were almost equally effective G-protein activators, DPH was considerably more effective than CPHE in RBL 2H3 cells (see Tables 1 and 2 and Fig. 5). Similarly, in human basophils and lung mast cells, DPH is a more effective stimulus than CPHE [5, 6]. Also noteworthy in this context is the finding that H_1-receptor antagonists showed disparate effects in the closely related Bt_2cAMP- and RA-differentiated HL-60 cells (see Figs. 1 and 4). Possibly, differences in the microenvironment of G-proteins account for the cell type-specific effects of H_1-receptor antagonists and other receptor-independent G-protein activators [10, 18].

In conclusion, we have shown that first-generation H_1-receptor antagonists possess the ability to activate G-proteins in a receptor-independent manner. Such a mode of action, and not cytotoxicity, presumably explains the stimulatory effects of these compounds in HL-60 cells, basophils, and mast cells.

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References

1. Rimmer SJ and Church MK, The pharmacology and mechanisms of action of histamine H_1-antagonists. *Clin Exp Allergy* **20**(Suppl. 2): 3–17, 1990.
2. Simons FE and Simons KJ, The pharmacology and use of H_1-receptor-antagonist drugs. *N Engl J Med* **330:** 1663–1670, 1994.
3. Mota I and Da Silva WD, The anti-anaphylactic and histamine-releasing properties of the antihistamines. Their effect on the mast cells. *Br J Pharmacol* **15:** 396–404, 1960.
4. Mota I, Release of histamine from mast cells. *Handb Exp Pharmacol* **18:** 569–636, 1966.
5. Lichtenstein LM and Gillespie E, The effects of the H1 and H2 antihistamines on "allergic" histamine release and its inhibition by histamine. *J Pharmacol Exp Ther* **192:** 441–450, 1975.
6. Church MK and Gradidge CF, Inhibition of histamine release from human lung *in vitro* by antihistamines and related drugs. *Br J Pharmacol* **69:** 663–667, 1980.
7. Mousli M, Bueb J-L, Bronner C, Rouot B and Landry Y, G protein activation: A receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides. *Trends Pharmacol Sci* **11:** 358–362, 1990.
8. Mousli M, Bronner C, Landry Y, Bockaert J and Rouot B, Direct activation of GTP-binding regulatory proteins (G-proteins) by substance P and compound 48/80. *FEBS Lett* **259:** 260–262, 1990.
9. Seifert R, Hagelüken A, Höer A, Höer D, Grünbaum L, Offermanns S, Schwaner I, Zingel V, Schunack W and Schultz G, The H_1 receptor agonist 2-(3-chlorophenyl)histamine activates G_i proteins in HL-60 cells through a mechanism that is independent of known histamine receptor subtypes. *Mol Pharmacol* **45:** 578–586, 1994.
10. Hagelüken A, Grünbaum L, Klinker JF, Nürnberg B, Harhammer R, Schultz G, Leschke C, Schunack W and Seifert R, Histamine receptor-dependent and/or independent activation of guanine nucleotide-binding proteins by histamine and 2-substituted histamine derivatives in human leukemia (HL-60) and human erythroleukemia (HEL) cells. *Biochem Pharmacol* **49:** 901–914, 1995.
11. Hagelüken A, Burde R, Nürnberg B, Harhammer R, Buschauer A and Seifert R, Cationic-amphiphilic arpromidine-derived guanidines and a histamine trifluoromethyl-toluidide derivative may activate pertussis toxin-sensitive G-proteins by a receptor-independent mechanism. *Naunyn-Schmiedeberg's Arch Pharmacol* **351:** 305–308, 1995.
12. Klinker JF, Hagelüken A, Grünbaum L, Heilmann I, Nürnberg B, Harhammer R, Offermanns S, Schwaner I, Ervens J, Wenzel-Seifert K, Müller T and Seifert R, Mastoparan may activate GTP hydrolysis by G_i-proteins in HL-60 membranes indirectly through interaction with nucleoside diphosphate kinase. *Biochem J* **304:** 377–383, 1994.
13. Warner JA, Yancey KB and MacGlashan DW Jr, The effect of pertussis toxin on mediator release from human basophils. *J Immunol* **139:** 161–165, 1987.
14. Bueb J-L, Mousli M, Bronner C, Rouot B and Landry Y, Activation of G_i-like proteins, a receptor-independent effect of kinins in mast cells. *Mol Pharmacol* **38:** 816–822, 1990.
15. Fischer T, Bronner C, Landry Y and Mousli M, The mechanism of inhibition of alkylamines on the mast-cell peptidergic pathway. *Biochim Biophys Acta* **1176:** 305–312, 1993.
16. Seifert R, Serke S, Huhn D, Bessler WG, Hauschildt S, Metzger J, Wiesmüller KH and Jung G, Incomplete functional differentiation of HL-60 leukemic cells by synthetic lipopeptides. Partial inhibition by pertussis toxin of enhanced superoxide formation. *Eur J Biochem* **203:** 143–151, 1992.
17. Barrett KE and Pearce FL, Heterogeneity of mast cells. *Handb Exp Pharmacol* **97:** 93–117, 1991.
18. Swieter M, Midura RJ, Nishikata H, Oliver C, Berenstein EH, Mergenhagen SE, Hascall VC and Siraganian RP, Mouse 3T3 fibroblasts induce rat basophilic leukemia (RBL-2H3) cells to acquire responsiveness to compound 48/80. *J Immunol* **150:** 617–624, 1993.
19. Higashijima T, Uzu S, Nakajima T and Ross EM, Mastoparan, a peptide toxin from wasp venom, mimics receptors by activating GTP-binding regulatory proteins (G proteins). *J Biol Chem* **263:** 6491–6494, 1988.
20. Jungblut PR and Seifert R, Analysis by high-resolution two-dimensional electrophoresis of differentiation-dependent alterations in cytosolic protein pattern of HL-60 leukemic cells. *J Biochem Biophys Meth* **21:** 47–58, 1990.
21. Offermanns S, Jones SVP, Bombien E and Schultz G, Stimulation of mitogen-activated protein kinase activity by different secretory stimuli in rat basophilic leukemia cells. *J Immunol* **152:** 250–261, 1994.
22. Seifert R and Schultz G, Reversible activation of NADPH oxi-

dase in membranes of HL-60 human leukemic cells. *Biochem Biophys Res Commun* **146:** 1296–1302, 1987.
23. Rosenthal W, Koesling D, Rudolph U, Kleuss C, Pallast M, Yajima M and Schultz G, Identification and characterization of the 35-kDa β-subunit of guanine-nucleotide-binding proteins by an antiserum raised against transducin. *Eur J Biochem* **158:** 255–263, 1986.
24. Seifert R, Höer A, Offermanns S, Buschauer A and Schunack W, Histamine increases cytosolic Ca^{2+} in dibutyryl-cAMP-differentiated HL-60 cells via H_1 receptors and is an incomplete secretagogue. *Mol Pharmacol* **42:** 227–234, 1992.
25. Wenzel-Seifert K and Seifert R, Nucleotide-, chemotactic peptide- and phorbol ester-induced exocytosis in HL-60 leukemic cells. *Immunobiology* **181:** 298–316, 1990.
26. Papermaster DS and Dreyer WJ, Rhodopsin content in the outer segment membranes of bovine and frog retinal rods. *Biochemistry* **13:** 2438–2444, 1974.
27. Kroll S, Phillips WJ and Cerione RA, The regulation of the cyclic GMP phosphodiesterase by the GDP-bound from of the α subunit of transducin. *J Biol Chem* **264:** 4490–4497, 1989.
28. Absolom DR, Basic methods for the study of phagocytosis. *Methods Enzymol* **132:** 95–180, 1986.
29. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
30. Walseth TF, Yuen PST and Moos MC Jr, Preparation of α-^{32}P-labeled nucleoside triphosphates, nicotinamide adenine dinucleotide, and cyclic nucleotides for use in determining adenylyl and guanylyl cyclases and cyclic nucleotide phosphodiesterase. *Methods Enzymol* **195:** 29–44, 1991.
31. Erbeck K, Klein JB and McLeish KR, Differential uncoupling of chemoattractant receptors from G proteins in retinoic acid-differentiated HL-60 granulocytes. *J Immunol* **150:** 1913–1921, 1993.
32. Lin P and Gilfillan AM, The role of calcium and protein kinase C in the IgE-dependent activation of phosphatidylcholine-specific phospholipase D in a rat mast (RBL 2H3) cell line. *Eur J Biochem* **207:** 163–168, 1992.
33. Tanimura A, Matsumoto Y and Tojyo Y, Mastoparan increases membrane permeability in rat parotid cells independently of action on G-proteins. *Biochem Biophys Res Commun* **177:** 802–808, 1991.
34. Demaurex N, Lew DP and Krause KH, Cyclopiazonic acid depletes intracellular Ca^{2+} stores and activates an influx pathway for divalent cations in HL-60 cells. *J Biol Chem* **267:** 2318–2324, 1992.
35. Krautwurst D, Seifert R, Hescheler J and Schultz G, Formyl peptides and ATP stimulate Ca^{2+} and Na^+ inward currents through non-selective cation channels via G-proteins in dibutyryl cyclic AMP-differentiated HL-60 cells. Involvement of Ca^{2+} and Na^+ in the activation of β-glucuronidase release and superoxide production. *Biochem J* **288:** 1025–1035, 1992.
36. Seifert R, Grünbaum L and Schultz G, Histamine H_1-receptors in HL-60 monocytes are coupled to G_i-proteins and pertussis toxin-insensitive G-proteins and mediate activation of Ca^{2+} influx without concomitant Ca^{2+} mobilization. *Naunyn-Schmiedeberg's Arch Pharmacol* **349:** 355–361, 1994.
37. Fasolato C, Hoth M, Matthews G and Penner R, Ca^{2+} and Mn^{2+} influx through receptor-mediated activation of nonspecific cation channels in mast cells. *Proc Natl Acad Sci USA* **90:** 3068–3072, 1993.
38. Hide M, Ali H, Price R, Moss J and Beaven MA, GTP-binding protein $G_{\alpha Z}$: Its down-regulation by dexamethasone and its credentials as a mediator of antigen-induced responses in RBL-2H3 cells. *Mol Pharmacol* **40:** 473–479, 1991.