Pergamon

Biochemical Pharmacology, Vol. 49, No. 7, pp. 901–914, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(94)00514-1

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

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(*Received* 25 *July* 1994; *accepted* 5 *November* 1994)

Abstract—In dibutyryl cAMP-differentiated human leukemia (HL-60) cells, the potent histamine H_1-receptor agonist, 2-(3-chlorophenyl)histamine, activates pertussis toxin (PTX)-sensitive guanine nucleotide-binding proteins (G-proteins) of the G_i-subfamily by a mechanism which is independent of known histamine receptor subtypes (Seifert *et al. Mol Pharmacol* **45**: 578–586, 1994). In order to learn more about this G-protein activation, we studied the effects of histamine and various 2-substituted histamine derivatives in various cell types and on purified G-proteins. In HL-60 cells, histamine and 2-methylhistamine increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in a clemastine-sensitive manner. Phenyl- and thienyl-substituted histamines increased $[Ca^{2+}]_i$ as well, but their effects were not inhibited by histamine receptor antagonists. 2-Substituted histamines activated high-affinity GTPase in HL-60 cell membranes in a PTX-sensitive manner, with the lipophilicity of substances increasing their effectiveness. Although HEL cells do not possess histamine receptors mediating rises in $[Ca^{2+}]_i$, 2-(3-bromophenyl)histamine increased $[Ca^{2+}]_i$ in a PTX-sensitive manner. It also increased GTP hydrolysis by G_i-proteins in HEL cell membranes. All these stimulatory effects of 2-substituted histamine derivatives were seen at concentrations higher than those required for activation of H_1-receptors. In various other cell types and membrane systems, 2-substituted histamine derivatives showed no or only weak stimulatory effects on G-proteins. 2-Substituted histamine derivatives activated GTP hydrolysis by purified bovine brain G_i/G_o-proteins and by pure G_{i2} (the major PTX-sensitive G-protein in HL-60 and HEL cells). Our data suggest the following: (1) histamine and 2-methylhistamine act as H_1-receptor agonists in HL-60 cells; (2) incorporation of bulky and lipophilic groups results in loss of H_1-agonistic activity of 2-substituted histamine derivatives in HL-60 cells but causes a receptor-independent G-protein-stimulatory activity; (3) the effects of 2-substituted histamine derivatives on G-proteins are cell-type specific.

Key words: cytosolic Ca^{2+} concentration; G-proteins; GTPase; histamine H_1-receptor; human myeloid cells; pertussis toxin; 2-substituted histamine derivatives

Human leukemia (HL-60) cells are pluripotent promyelocytes which can be differentiated towards monocytes with $1\alpha,25(OH)_2D_3$§ [1] or towards neutrophils with DMSO or Bt_2cAMP [2, 3]. Differentiation of HL-60 cells is associated with the expression of formyl peptide receptors and the superoxide anion-generating NADPH oxidase [EC 1.6.99.6.] [1–3]. Formyl peptide receptors interact with G-proteins of the G_i-subfamily which mediate activation of phospholipase C [EC 3.1.4.10] with a subsequent rise in $[Ca^{2+}]_i$ and stimulation of superoxide anion formation [1, 2, 4]. G_i- and G_o-proteins are substrates for the exotoxin of *Bordetella pertussis*, PTX, which mediates ADP-ribosylation of a cysteine residue near the C-terminus of α-subunits [5].

In addition to formyl peptide receptors, Bt_2cAMP-differentiated and $1\alpha,25(OH)_2D_3$-differentiated HL-60 cells possess histamine H_1-receptors coupled to G_i-proteins and as yet unidentified PTX-insensitive G-proteins [1, 2]. Like formyl peptide receptors, H_1-receptors mediate increases in $[Ca^{2+}]_i$ [1, 2]. In a recent study, we have shown that 2-(3-chlorophenyl)histamine, a potent full H_1-receptor agonist in the guinea pig ileum [6], increases $[Ca^{2+}]_i$

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§ Abbreviations: Bt_2cAMP, dibutyryl cAMP; $[Ca^{2+}]_i$, cytosolic Ca^{2+} concentration; G-protein, guanine nucleotide-binding protein; GTP[γS], guanosine 5-*O*-[3-thio]-triphosphate; MP, mastoparan (INLKALAALAKKIL); NDPK, nucleoside diphosphate kinase; NEM, *N*-ethylmaleinimide; $1\alpha,25(OH)_2D_3$, $1\alpha,25$-dihydroxycholecalciferol; PTX, pertussis toxin.

in Bt_2cAMP-differentiated HL-60 cells in a PTX-sensitive manner [4]. In addition, the 2-substituted histamine derivative increases PTX-sensitive high-affinity GTPase [EC 3.6.1.-] activity and GTP[γS] binding in HL-60 cell membranes [4]. Moreover, 2-(3-chlorophenyl)histamine activates photolabeling of G_i-protein α-subunits with GTP azidoanilide in this system. Unlike histamine, 2-(3-chlorophenyl)-histamine activates tyrosine phosphorylation and superoxide anion formation in HL-60 cells, and its stimulatory effects are resistant to inhibition by H_1-, H_2- and H_3-receptor antagonists [4]. These data indicate that 2-(3-chlorophenyl)histamine activates G_i-proteins in HL-60 cells by a mechanism which is independent of known histamine receptor subtypes. Interestingly, Leurs *et al.* [7] recently showed that histamine and 2-methylhistamine on one hand, and 2-phenylhistamines on the other, interact with different sites of the H_1-receptor molecule. Specifically, hydrophobic domains may be of importance for the interaction of 2-phenylhistamines with H_1-receptors.

Mast cells are another model system for the analysis of receptor-independent G-protein activation. Various cationic–amphiphilic substances such as compound 48/80, substance P and the wasp venom, MP, induce mast cell activation via G_i-proteins in a receptor-independent manner [8, 9]. All these substances also activate purified G-proteins directly [8–10]. MP activates G-proteins in various cell types whereas the effects of compound 48/80 appear to be restricted to mast cells [8, 9, 11–13]. Intriguingly, in HL-60 cells, MP may activate G-proteins largely through stimulation of NDPK [EC 2.7.4.6.], leading to enhanced formation and subsequent cleavage of GTP [14].

Several questions arise from the above-mentioned studies:

1. What are the structure–activity relationships of 2-substituted histamine derivatives with respect to histamine receptor-dependent and -independent G-protein activation?
2. Do 2-substituted histamine derivatives act as agonists at an as yet unknown histamine receptor subtype or at an orphan receptor, i.e. a receptor for which endogenous ligands are not yet known?
3. Like compound 48/80, substance P and MP, 2-(3-chlorophenyl)histamine and other 2-substituted histamine derivatives are cationic–amphiphilic substances [6, 15]. This raises the question as to whether they activate PTX-sensitive G-proteins in a receptor-independent manner.
4. Does NDPK contribute to G-protein activation induced by 2-substituted histamine derivatives?
5. What is the cell-type specificity of G-protein activation induced by 2-substituted histamine derivatives?

In order to examine these questions, we studied the effects of histamine and 18 2-substituted histamine derivatives in HL-60 cells, human erythroleukemia (HEL) cells, human neuroblastoma (SH-SY5Y) cells, rat basophilic leukemia (RBL 2H3) cells and hamster ductus deferens smooth muscle (DDT_1MF-2) cells. All cell lines studied express G_i-proteins, with SH-SY5Y cells additionally expressing G_o-proteins.

MATERIALS AND METHODS

Materials. Compounds **2–19** were synthesized as described [6, 15]. Stock solutions of histamine and 2-substituted histamine derivatives (10–100 mM each) were prepared in distilled water and stored at −20°. 1α,25$(OH)_2D_3$ was a gift from Drs A. Kaiser and U. Fischer (Hoffman-La Roche, Basel, Switzerland). MP was from Saxon Biochemicals (Hannover, Germany) and was dissolved in 1 mM sodium acetate, pH 5.0, and stored at −20°. PTX was from List Biological Laboratories (Campbell, CA, U.S.A.). NDPK (80 U/mg, activity measured at 25° using dTDP and ATP as substrates and purified from bovine liver mitochondria according to Glaze and Wadkins [16]) was purchased from Boehringer Mannheim (Mannheim, Germany). Sources of other materials have been described elsewhere [1–4, 14, 17–21].

Cell culture and membrane preparation. Cell lines were purchased from the American Type Culture Collection (Rockville, MD, U.S.A.). HL-60 promyelocytes were grown in suspension culture in RPMI-1640 medium supplemented with 10% (v/v) horse serum, 1% (v/v) non-essential amino acids, 2 mM L-glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin in a humidified atmosphere with 7% CO_2 at 37°. Cells were differentiated towards neutrophil-like cells with Bt_2cAMP (0.2 mM) for 48 hr or with DMSO (160 mM) for 120 hr [2, 3]. Monocytic differentiation was induced by treatment with 1α,25$(OH)_2D_3$ (10 nM) for 120 hr [1]. HEL cells were cultured as HL-60 promyelocytes except that fetal calf serum was employed. PTX (100 ng/mL) or its carrier (control) were added to cell cultures 24 hr before experiments or membrane preparation. Under these conditions, virtually all G_i-protein α-subunits were ADP-ribosylated as assessed by subsequent ADP-ribosylation of cell membranes with activated PTX and [^{32}P]NAD (data not shown). SH-SY5Y cells were grown as monolayers in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere with 7% CO_2 at 37°. RBL 2H3 and DDT_1MF-2 cells were grown as monolayers in DMEM medium supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine, 50 U/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere with 7% CO_2 at 37°. Cell membranes were prepared as described in [3].

Measurement of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was determined using the fluorescent dye, fura-2, as described in [2]. Fluorescence of cells (1.0×10^6 cells in 2 mL) was determined at 37° under constant stirring at 10^3 rpm using a Ratio II spectrofluorometer (Aminco, Silver Spring, MD, U.S.A.). Cells were incubated for 3 min in the presence of various substances before the addition of stimuli. The excitation and emission wavelengths were 340 and 510 nm, respectively.

Purification of G-proteins. Heterotrimeric G_i/G_o-proteins were purified from bovine brain membranes as described [17]. The preparation of G_i/G_o-proteins (purity >90%) contained predominantly G_{o1}, significant amounts of G_{o2} and of another yet unidentified G_o-subtype ("G_{o3}"), G_{i1} and G_{i2} and

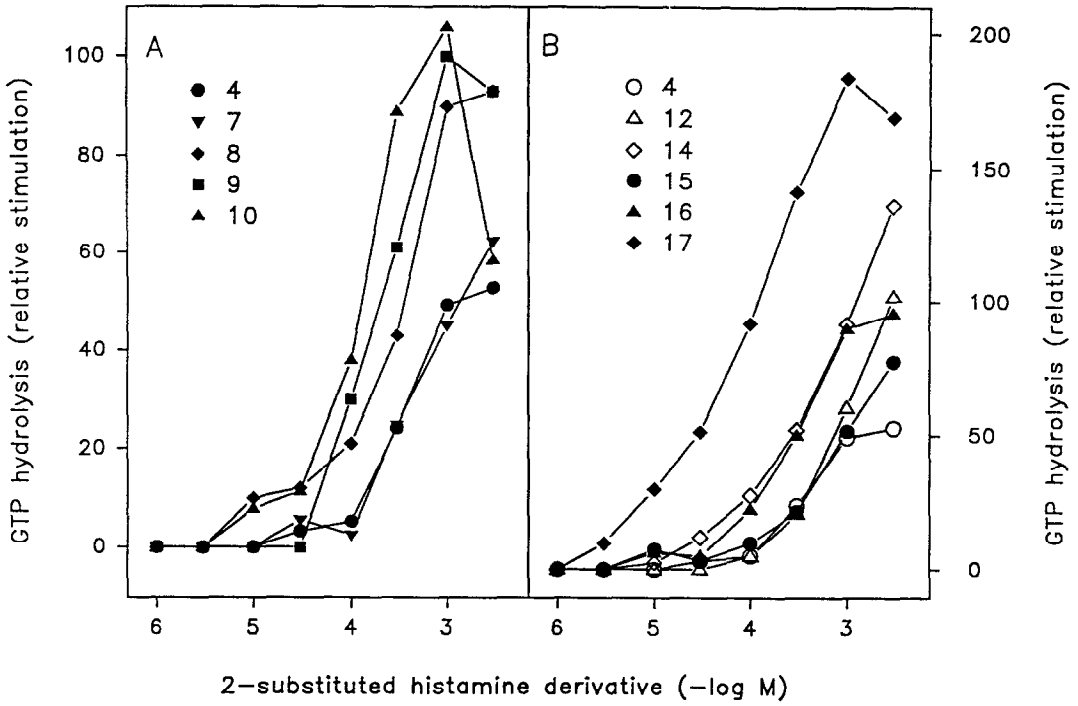


Fig. 1. Concentration–response curves of the stimulatory effects of various 2-substituted histamine derivatives on GTP hydrolysis in HL-60 cell membranes. GTP hydrolysis in membranes of Bt_2cAMP-differentiated HL-60 cells was determined in the presence of 2-substituted histamine derivatives at the indicated concentrations as described in Materials and Methods. The increase in GTP hydrolysis caused by compound **9** (1 mM) was defined as 100%. The relative stimulatory effects of other substances are referred to this value. Symbols in (A) and (B) designate the curves for the various compounds. Data shown are the means of three independent experiments performed in quadruplicate. The SD values were generally <5% of the means.

traces of G_{i3}. Heterotrimeric G_{i2} was purified from bovine brain membranes as described in [17]. Reconstitution of G-proteins into phospholipid vesicles was performed according to Hagelüken *et al.* [18]. Briefly, G-proteins (25–30 pmoles) were mixed with azolectin (0.1%, w/v) and sodium cholate (1%, w/v) in a buffer consisting of 100 mM NaCl, 2 mM $MgCl_2$, 1 mM EDTA and 20 mM HEPES/NaOH, pH 8.0, 4°, and loaded onto a 10 mL AcA 34 gel filtration column (25 cm × 8.5 mm) equilibrated with the above buffer. Liposomes eluted in the void volume. Association of G-proteins to liposomes was confirmed by [^{35}S]GTP[γS] binding [17]. Pooled fractions were then used for measurement of GTP hydrolysis.

GTPase assay. GTP hydrolysis was determined as described in [4]. For determination of GTP hydrolysis in cell membranes, reaction mixtures (100 μL) contained 3.0–15.0 μg of protein/tube, 0.5 μM [γ-^{32}P]GTP (0.1 μCi/tube), 0.5 mM $MgCl_2$, 0.1 mM EGTA, 0.1 mM ATP, 1 mM adenosine 5′-[β,γ-imido]triphosphate, 5 mM creatine phosphate, 40 μg of creatine kinase, 1 mM dithiothreitol and 0.2% (w/v) BSA in 50 mM triethanolamine/HCl, pH 7.4, and substances at various concentrations. Reactions were conducted for 15 min at 25°. Low-affinity GTPase activity was determined in the presence of GTP (50 μM) and was <5% (HL-60 cell membranes), <15% (SH-SY5Y and RBL 2H3 cell membranes), <20% (HEL cell membranes) and <25% (DDT_1MF-2 cell membranes) of GTP hydrolysis measured in the presence of 0.5 μM GTP. For determination of the GTPase activity of purified G-proteins, reaction mixtures (100 μL) contained 0.4–0.6 pmoles of G_i/G_o-proteins or of G_{i2}, 50 nM [γ-^{32}P]GTP, 1.0 mM $MgCl_2$, 0.8 mM EDTA and 30 mM NaCl. The other assay conditions were as described above.

Assay for GTP formation. For determination of [^{3}H]GTP formation in HL-60 cell membranes, reaction mixtures (50 μL) contained 0.5 μg of membrane protein/tube, 0.5 μM [^{3}H]GDP (1 μCi/tube), 10 μM GTP, 0.5 mM $MgCl_2$, 0.1 mM EGTA, 1 mM adenosine 5′-[β,γ-imido]triphosphate, 5 mM creatine phosphate, 40 μg of creatine kinase, 1 mM dithiothreitol and 0.2% (w/v) BSA in 50 mM triethanolamine/HCl, pH 7.4 [14]. Reactions were conducted for 10 min at 25°. The stopping of reactions, separation of nucleotides by TLC and nucleotide elution from TLC plates were performed as described in [19]. For determination of [^{3}H]GTP formation by the purified enzyme, reaction mixtures (50 μL) contained 0.1 mU of NDPK from bovine liver mitochondria, 0.5 μM [^{3}H]GDP (1 μCi/tube), 10 μM GTP and 5 mM $MgCl_2$ in 50 mM triethanolamine/HCl, pH 7.4 [14]. Reactions were conducted from 10 min at 25°.

Miscellaneous. Human neutrophils from healthy volunteers were isolated as described in [20].

Table 1. Effects of histamine and various 2-substituted histamine derivatives at H_1-receptors in the guinea pig ileum, on GTP hydrolysis in HL-60 cell membranes, on increases in $[Ca^{2+}]_i$ in HL-60 cells and on GTP hydrolysis by purified G_i/G_o-proteins

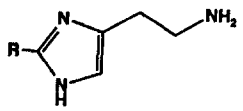
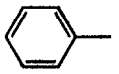
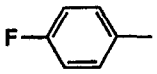
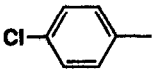
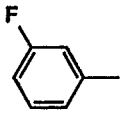
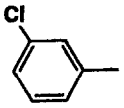
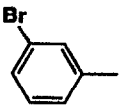
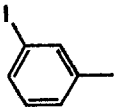
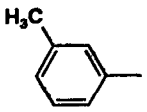
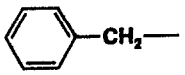
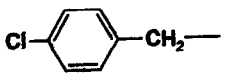
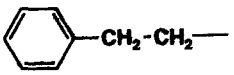
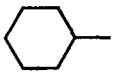
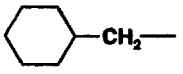
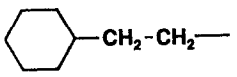
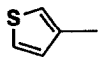
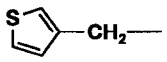
No.	 R	H_1-receptor (guinea pig ileum) i.a.	EC_{50} (μM)	K_B (μM)	GTP hydrolysis (HL-60 membranes) rel. eff. (%)	EC_{50} (mM)	Increase in $[Ca^{2+}]_i$ (HL-60 cells) rel. eff. (%)	GTP hydrolysis (G_i/G_o-proteins) rel. eff. (%)
1	H—	1.0	0.20		18		172	0
2	H_3C—	1.0	1.41		33		70	3
3	H_3C-CH_2—	1.0	2.88		30		0	3
4		1.0	1.55		45	0.32	97	67
5		1.0	1.41		44		126	70
6		0.5		12.6	100		0	40
7		1.0	0.23		48		114	67
8		1.0	0.24		94	0.32	112	13
9		1.0	0.18		100	0.20	100	100
10		1.0	0.22		105	0.16	173	105
11		1.0	0.66		62	0.16	116	68
12		0.9	6.61		61		25	18
13		0.4	5.01		95	0.20	0	52
14		0.9	6.61		93		0	28
15		0.2		1.58	53		0	18
16		0.1		1.99	82	0.32	0	30

Table 1 (continued)

No.	R	H_1-receptor (guinea pig ileum) i.a.	EC_{50} (μM)	K_B (μM)	GTP hydrolysis (HL-60 membranes) rel. eff. (%)	EC_{50} (mM)	Increase in $[Ca^{2+}]_i$ (HL-60 cells) rel. eff. (%)	GTP hydrolysis (G_i/G_o-proteins) rel. eff. (%)
17		0		5.01	185	0.10	0	50
18		1.0	0.30		43		116	65
19		1.0	5.01		50		87	52

H_1-receptors: the values of the intrinsic activities (i.a.) and potencies (EC_{50}) of compounds **1–9, 11, 12, 14** and **16–19** at H_1-receptors in the guinea pig ileum were taken from Dziuron and Schunack [15], Zingel *et al.* [6] and Zingel and Schunack [24]. The corresponding values of compounds **10, 13** and **15** were determined as described by Zingel *et al.* [6]. K_B values (antagonist equilibrium dissociation constants) of compounds **15–17** were taken from Dziuron and Schunack [15]. The K_B value of compound **6** was determined as described [15].

GTP hydrolysis: GTP hydrolysis in membranes of Bt_2cAMP-differentiated HL-60 cells was determined in the presence of compounds **1–19** (1 mM each) or solvent (control) as described in Materials and Methods. The increase in GTP hydrolysis caused by compound **9** was defined as 100%. The relative effectiveness (rel. eff.) of compounds **1–8** and **10–19** (1 mM each) is referred to this value. EC_{50} values were calculated only for those substances where stimulatory effects on GTPase reached saturation up to 3 mM. Data shown are the means of at least three independent experiments performed in quadruplicate. The SD values were generally <5% of the means. GTP hydrolysis by G_i/G_o-proteins was determined in the presence of compounds **1–19** (5 mM each) or solvent (control) as described in Materials and Methods. The increase in GTP hydrolysis caused by compound **9** was defined as 100%. The relative effectiveness (rel. eff.) of compounds **1–8** and **10–19** is referred to this value.

Increase in $[Ca^{2+}]_i$: HL-60 cells were harvested, loaded with fura-2-acetoxymethylester, and the stimulatory effects of compounds **1–19** (100 μM each) on $[Ca^{2+}]_i$ were assessed as described in Materials and Methods. The increase in $[Ca^{2+}]_i$ caused by compound **9** (225 ± 34 nM, N = 4) was defined as 100%. The relative effectiveness (rel. eff.) of compounds **1–8** and **10–19** is referred to this value.

Pretreatment of HL-60 cell membranes with NEM was performed as described in [21]. Protein was determined according to Lowry *et al.* [22]. [γ-^{32}P]-GDP was prepared as described in [23].

RESULTS

Figure 1 shows the concentration–response curves of the stimulatory effects of some 2-substituted histamine derivatives on high-affinity GTP hydrolysis in membranes of Bt_2cAMP-differentiated HL-60 cells. Table 1 shows the structural formulae of the substances studied and summarizes their effects on GTP hydrolysis in HL-60 cell membranes. For the data shown in Fig. 1 and Table 1, the stimulatory effect of compound **9** (1 mM) on GTP hydrolysis was defined as 100%, and the stimulatory effects of other substances are referred to this value. Stimulation of GTP hydrolysis in HL-60 cell membranes by compounds **4**, **8–11**, **13**, **16** and **17** reached saturation up to 3 mM (EC_{50} values ranging from 0.10–0.32 mM). The effects of the other substances did not reach saturation and EC_{50} values could therefore not be calculated.

Histamine at a concentration of 1 mM showed a stimulatory effect on GTPase (see Table 1), but at 1–100 μM, i.e. concentrations commonly needed for activation of H_1-, H_2- and H_3-receptors, histamine was ineffective (data not shown). Incorporation of a methylene or ethylene group (compounds **2** and **3**) led to a small increase in effectiveness in activating GTP hydrolysis. The effectiveness of substances was further enhanced by introduction of a phenyl ring (**4**). Substitution of the phenyl ring with bulky halogens in the *meta* position, e.g. chlorine (**8**), bromine (**9**) or iodine (**10**), substantially increased the effectiveness of the substances to activate GTP hydrolysis (compare with **4**). In addition, there was an inverse correlation between electronegativity of halogens and the effectiveness of halogen-substituted histamine derivatives to activate GTPase in HL-60 cell membranes (**7–10**). Incorporation of less bulky substituents, e.g. fluorine (**7**) or a methyl group (**11**), resulted in smaller increases in effectiveness of substances to activate GTP hydrolysis. Whether halogenation was in the *meta* or *para* position of the phenyl ring (compare **5** with **7** and **6** with **8**) was not significant.

The type of ring system connected to imidazole influenced GTPase activation in HL-60 cell membranes as well. In the case of derivatives with a methylene bridge, the substance possessing a cyclohexyl group (**16**) was more effective than the one with a phenyl group (**12**) which, in turn, was more effective than the one with a thienyl group (**19**). When compounds with an ethylene bridge are

Table 2. Effects of various 2-substituted histamine derivatives on $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells

Stimulus	Increase in $[Ca^{2+}]_i$ (nM) control	clemastine	famotidine
Compound **2**	155 ± 21	0	143 ± 16
Compound **3**	0	0	0
Compound **4**	213 ± 34	218 ± 20	222 ± 24
Compound **9**	225 ± 34	235 ± 21	218 ± 17
Compound **19**	193 ± 22	212 ± 21	214 ± 23

HL-60 cells were harvested, loaded with fura-2-acetoxymethylester, and the effects of compound **2–4, 9** and **19** (100 μM each) on $[Ca^{2+}]_i$ were assessed as described in Materials and Methods. Three minutes before the addition of stimuli, clemastine or famotidine (10 μM each) or solvent (control) was added to cells. Clemastine and famotidine by themselves had no effect on $[Ca^{2+}]_i$ (data not shown). Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.

considered, the difference in effectiveness between phenyl- and cyclohexyl-substituted derivatives becomes even more evident (compare **14** and **17**).

The length of the alkyl chain between imidazole and the ring system was also of importance for GTPase activation. A methylene group and, even more prominently, an ethylene group increased the effectiveness of cyclohexyl and phenyl derivatives to activate GTP hydrolysis (compare **15–17** and **4**, **12** and **14**). Similar to compounds **4** and **6**, chlorination of compound **12** (resulting in **13**), led to a further increase in effectiveness of substances to activate GTP hydrolysis.

The stimulatory effects of compound **8** on GTPase in HL-60 cell membranes are PTX-sensitive [4]. By analogy to compound **8**, the stimulatory effects of the other 2-substituted histamine derivatives were abolished in membranes obtained from PTX-treated Bt_2cAMP-differentiated HL-60 cells (data not shown), indicative of G_i-protein activation. PTX abolished the stimulatory effect of histamine on GTP hydrolysis as well (data not shown).

Table 1 also summarizes the effects of histamine and 2-substituted histamine derivatives on $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells. Among all substances studied, histamine and compound **10** were the most effective in increasing $[Ca^{2+}]_i$, followed by a group of compounds with intermediate stimulatory activity (**4**, **5**, **7–9**, **11**, **18** and **19**). Compound **12** increased $[Ca^{2+}]_i$ only marginally, and compounds **3**, **6** and **13–17** were ineffective. As has been shown for the stimulatory effects of compound **8** on $[Ca^{2+}]_i$ [4], the effects of compounds **4**, **5**, **7–12**, **18** and **19** were abolished by PTX (data not shown).

Next, we studied the effects of the H_1-receptor antagonist, clemastine, and the H_2-receptor antagonist, famotidine, on rises in $[Ca^{2+}]_i$ and GTPase activation. The stimulatory effect of 2-methylhistamine (**2**) on $[Ca^{2+}]_i$ was inhibited by clemastine but not by famotidine (Table 2). Neither clemastine nor famotidine had an inhibitory effect on rises in $[Ca^{2+}]_i$ induced by compounds **4**, **9** and **19**. In addition, the stimulation of GTP hydrolysis caused by these substances was insensitive to the aforementioned receptor antagonists (data not shown).

Clemastine shows non-competitive i.e. unsurmountable, antagonism at H_1-receptors in Bt_2cAMP-differentiated HL-60 cells [2]. In accordance with these data, clemastine (10 μM) abolished the effect of histamine (3 mM) on $[Ca^{2+}]_i$ in these cells (Table 3). In contrast, clemastine had no inhibitory effect on histamine-stimulated GTP hydrolysis in HL-60 cell membranes. This was also true for famotidine.

In order to assess the role of NDPK in G-protein activation in membranes of Bt_2cAMP-differentiated HL-60 cells induced by 2-substituted histamine derivatives, we studied the effects of compounds **6** and **9** (10 μM–1 mM) on GTP formation catalysed by NDPK in HL-60 cell membranes and by purified NDPK. Whereas MP increased GTP formation in HL-60 membranes and the pure enzyme by approximately 40% and 150%, respectively [14], the

Table 3. Stimulatory effects of histamine on $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells and on GTP hydrolysis in membranes of Bt_2cAMP-differentiated HL-60 cells: differential inhibition by clemastine

Stimulus	$[Ca^{2+}]_i$ (nM)	GTP hydrolysis (pmol/mg/min)
None	121 ± 13	21.7 ± 1.6
Histamine	422 ± 25	26.5 ± 1.3 (22.1%)
Histamine + clemastine	119 ± 14	26.8 ± 1.8 (23.5%)
Histamine + famotidine	426 ± 35	26.7 ± 1.7 (23.0%)

HL-60 cells were harvested, loaded with fura-2-acetoxymethylester, and the rise in $[Ca^{2+}]_i$ induced by histamine (3 mM) was assessed as described in Materials and Methods. Three minutes before the addition of stimuli, clemastine or famotidine (10 μM each) or solvent was added to cells. GTP hydrolysis in HL-60 cells membranes was measured in the absence or presence of histamine (3 mM). Reaction mixtures additionally contained clemastine or famotidine (10 μM each) or solvent. At the concentrations employed, clemastine and famotidine by themselves had no effect on GTP hydrolysis. Numbers in parentheses indicate the relative stimulatory effects of histamine referred to basal GTP hydrolysis. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.

2-substituted histamine derivatives showed no significant stimulatory effects (data not shown).

We also studied the interaction of compound **8** with MP on GTP hydrolysis in HL-60 cell membranes. In control membranes, compound **8** and MP at maximally effective concentrations increased GTP hydrolysis by approximately 70% and 100%, respectively (Table 4). In combination, the two substances increased GTP hydrolysis by approximately 130%, i.e. in a subadditive manner. NEM alkylates G_i-protein α-subunits and, in a manner similar to that of PTX, uncouples formyl peptide receptors from G_i-proteins [21]. Pretreatment of HL-60 cell membranes with NEM reduced the stimulatory effects of compound **8** and MP by more than 60%. Despite this reduction, compound **8** and MP interacted in a synergistic manner to increase GTP hydrolysis in NEM-treated HL-60 cell membranes (see Table 4).

Next, we studied the effects of 2-substituted histamine derivatives on G-protein activation in various human myeloid cell types. As in Bt_2cAMP-differentiated HL-60 cells, compound **7** increased $[Ca^{2+}]_i$ in HL-60 promyelocytes in a PTX-sensitive manner (Table 5). The lower effectiveness of compound **7** in HL-60 promyelocytes compared to Bt_2cAMP-differentiated HL-60 cells may be due to a lower concentration of G_i-proteins in the former [25]. The stimulatory effects of compound **7** on $[Ca^{2+}]_i$ in these cells were not affected by H_1- and H_2-receptor antagonists (data not shown). Unexpectedly, however, compound **7** did not induce rises in $[Ca^{2+}]_i$ in DMSO and $1\alpha,25(OH)_2D_3$-differentiated HL-60 cells and human neutrophils (see Table 5). Similar data to that shown in Table 5 with compound **7** were obtained with compound **8** (not shown).

Table 6 compares the effects of compound **7** and MP on GTP hydrolysis in membranes from HL-60 promyelocytes and DMSO-differentiated HL-60 cells. Basal GTP hydrolysis in membranes from undifferentiated cells was considerably lower than in membranes from DMSO-differentiated HL-60 cells. This finding is in accordance with the fact that the concentration of G_i-proteins in HL-60 promyelocytes is lower than in differentiated HL-60 cells [25]. Compound **7** and MP increased GTP hydrolysis in both systems, their stimulatory effects in membranes of differentiated cells being greater than in membranes from promyelocytes (see Table 6).

In HEL cells, compound **9** (100 μM) induced a rapid increase in $[Ca^{2+}]_i$ which declined to a plateau above basal values within 2 min (Fig. 2A). The kinetics of this increase in $[Ca^{2+}]_i$ resembled those for 2-substituted histamine derivatives in Bt_2cAMP-

Table 4. Interaction of compound **8** with MP on GTP hydrolysis in membranes of Bt_2cAMP-differentiated HL-60 cells: effect of treatment with NEM

Stimulus	GTP hydrolysis (pmol/mg/min) control	NEM
None	15.3 ± 0.9	11.5 ± 0.3
Compound **8**	26.0 ± 0.5 (69.9%)	13.2 ± 0.7 (14.8%)
MP	30.3 ± 1.1 (98.0%)	15.6 ± 0.6 (35.6%)
Compound **8** + MP	35.5 ± 0.8 (132.0%)	20.2 ± 0.8 (75.7%)

Treatment of HL-60 cell membranes with NEM or solvent (control) was performed as described in Materials and Methods. GTP hydrolysis was determined in the absence or presence of compound **8** (1 mM) and/or MP (10 μM). Numbers in parentheses indicate the relative stimulatory effects of substances referred to basal GTP hydrolysis. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.

Table 5. Effects of compound **7** on $[Ca^{2+}]_i$ in various human myeloid cell types

Cell type	Increase in $[Ca^{2+}]_i$ (nM) control	PTX
HL-60 promyelocytes	85 ± 28	0
Bt_2cAMP-differentiated HL-60 cells	202 ± 25	0
DMSO-differentiated HL-60 cells	0	n.d.
$1\alpha,25(OH)_2D_3$-differentiated HL-60 cells	0	n.d.
Human neutrophils	0	n.d.

HL-60 cells were subjected to various differentiations and treatment with PTX as described in Materials and Methods. Human neutrophils were isolated as described in Materials and Methods. Cells were loaded with fura-2-acetoxymethylester, and the effects of compound **7** (100 μM) on $[Ca^{2+}]_i$ were assessed as described in Materials and Methods. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments; n.d., not determined.

Table 6. Effects of compound 7 and MP on GTP hydrolysis in membranes of HL-60 promyelocytes and DMSO-differentiated HL-60 cells

None	GTP hydrolysis (pmol/mg/min) HL-60 promyelocytes	DMSO-differentiated HL-60 cells
Solvent (control	12.1 ± 0.5	27.8 ± 1.4
Compound **7**	13.7 ± 0.5 (13.2%)	37.8 ± 1.4 (40.0%)
MP	15.1 ± 0.6 (24.8%)	45.9 ± 1.7 (71.9%)

GTP hydrolysis in HL-60 membranes was determined in the absence or presence of compound **7** (1 mM) or MP (10 μM) as described in Materials in Methods. Numbers in parentheses indicate the relative stimulatory effects of substances referred to basal GTP hydrolysis. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.

differentiated HL-60 cells [4] and HL-60 promyelocytes (data not shown). However, unlike in the latter two cell types [2, 26], histamine did not induce rises in $[Ca^{2+}]_i$ in HEL cells (see Fig. 2A) [26, 27]. Pretreatment of HEL cells with PTX abolished the stimulatory effect of compound **9** on $[Ca^{2+}]_i$ (see Fig. 2A). In HEL cell membranes, compound **9** (1 mM) increased GTP hydrolysis by approximately 60% and was ineffective in membranes from PTX-treated cells (Fig. 2B).

Figure 3 compares the effects of various 2-substituted histamine derivatives (1 mM each) on GTP hydrolysis in HEL cell membranes. Like in HL-60 cell membranes (see Table 1), incorporation of a bulky halogen into the phenyl ring (compare **4** with **8** and **9**) enhanced the GTPase-activating properties of the substances. In addition, the order of effectiveness of substances **15–17** in both systems is similar. However, compared to HL-60 cell membranes, compounds **12** and **14–17** were only weak activators of GTPase in HEL cell membranes.

When the effects of compound **9** and MP on GTP hydrolysis in HEL cell membranes, RBL 2H3 cell membranes, SH-SY5Y cell membranes and DDT_1MF-2 cell membranes were compared, prominent differences became apparent (Fig. 4). With

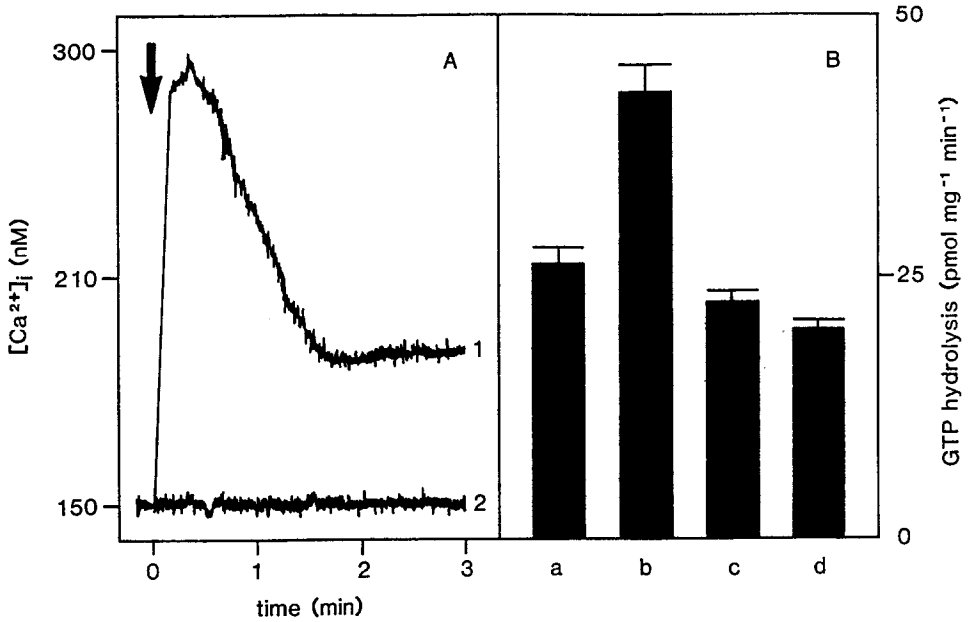


Fig. 2. Effects of compound **9** and histamine on $[Ca^{2+}]_i$ in HEL cells and of compound **9** on GTP hydrolysis in HEL cell membranes. HEL cells were treated with PTX or carrier (control) as described in Materials and Methods. (A) Time courses of rises in $[Ca^{2+}]_i$. HEL cells were harvested, loaded with fura-2-acetoxymethylester, and the effects of compound **9** or histamine (100 μM each) on $[Ca^{2+}]_i$ were assessed. Trace 1, compound **9** in control cells; trace 2, compound **9** in PTX-treated cells or histamine in control cells. The arrow indicates the addition of compound **9** or histamine. Superimposed original tracings from a representative experiment are shown. Similar results were obtained in three independent experiments. (B) GTP hydrolysis in HEL cell membranes. GTP hydrolysis in cell membranes of control cells (a and b) and of PTX-treated cells (c and d) was determined as described in Materials and Methods in the presence of solvent (basal activity) (a and c) or compound **9** (1 mM) (b and d). Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.

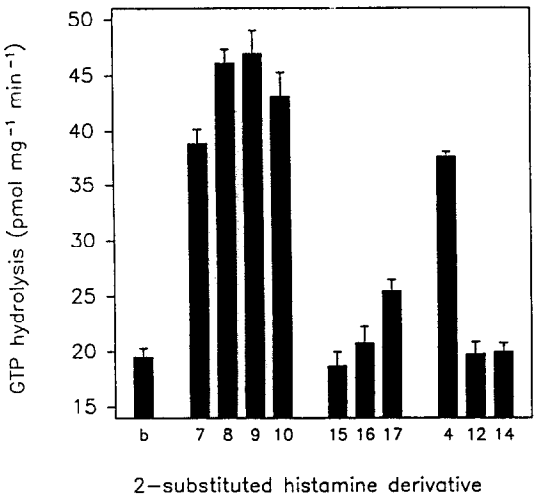


Fig. 3. Effects of various 2-substituted histamine derivatives on GTP hydrolysis in HEL cell membranes. GTP hydrolysis in HEL cell membranes was determined in the presence of various 2-substituted histamine derivatives (1 mM each) as described in Materials and Methods. Numbers below columns designate the corresponding compounds; b, basal GTP hydrolysis. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.

respect to MP, the substance was effective in activating GTP hydrolysis in the latter three systems but was quite ineffective in the former. With regard to compound **9**, the opposite is true. In membranes of SH-SY5Y cell membranes, compound **9** increased GTP hydrolysis only up to 15%. In membranes of DDT_1MF-2 cells, the substance was virtually without effect, and in membranes of RBL 2H3 cells, compound **9** even showed inhibitory effects on GTP hydrolysis.

Figure 5 shows concentration–response curves of the stimulatory effects of compound **4** on GTP hydrolysis by purified G_i/G_o-proteins and by pure G_{i2}, the latter being the major PTX-sensitive G-protein in HL-60 and HEL cells [25, 27, 28]. Compound **4** increased GTP hydrolysis by the purified G-proteins with an EC_{50} of approximately 5–6 mM and a maximum at 10 mM. The rank order of effectiveness of histamine and 2-substituted histamine derivatives (5 mM each) to activate GTP hydrolysis by purified G_i/G_o-proteins is summarized in Table 1. Histamine did not increase GTP hydrolysis by purified G-proteins. Among 2-substituted histamine derivatives, compounds **9** and **10** were the most effective GTPase activators, followed by a group of substances with intermediate (**4**, **5**, **7**, **11**, **13**, **17–19**) and low stimulatory activity (**2**, **3**, **6**, **8**, **12**, **14–16**).

DISCUSSION

Histamine and 2-methylhistamine (**2**) increased $[Ca^{2+}]_i$ in Bt_2cAMP-differentiated HL-60 cells in a clemastine-sensitive manner (see Tables 2 and 3). These findings indicate that histamine and 2-methylhistamine act as H_1-receptor agonists in HL-60 cells as is the case for other systems [7, 24]. The incorporation of an ethylene group into the molecule led to a loss of stimulatory activity on $[Ca^{2+}]_i$ (see Table 1). Interestingly, the incorporation of a more bulky and lipophilic substituent, i.e. a phenyl or a thienyl ring, resulted in the reappearance of the stimulatory effect of 2-substituted histamine derivatives on $[Ca^{2+}]_i$ (see Table 1). However, these effects were no longer sensitive to blockade by H_1- and H_2-receptor antagonists (see Table 2). Our present findings support the recent suggestion by Leurs *et al.* [7] that the mechanism by which 2-phenylhistamines interact with H_1-receptors is different from that of histamine and 2-methylhistamine. Specifically, as the receptor reserve for H_1-receptors in Bt_2cAMP-differentiated HL-60 cells is apparently very small [4], a potential stimulatory effect of 2-phenylhistamines at these receptors may be masked by effective interaction of the compounds with a hydrophobic site which is different from H_1-receptors (see below).

The pattern of effectiveness of 2-substituted histamine derivatives in activating GTP hydrolysis in HL-60 cell membranes and exerting agonistic effects at H_1-receptors in the guinea pig ileum is quite different (see Table 1). For example, compound **8** is a potent full H_1-receptor agonist in the guinea pig ileum, and compound **6** is a partial agonist. However, with respect to GTPase activation in HL-60 cell membranes, compounds **6** and **8** showed similar stimulatory effects. In addition, cyclohexyl derivatives (**15–17**) are H_1-receptor antagonists but concerning GTP hydrolysis in HL-60 cell membranes, they are stimulatory. Moreover, histamine, the endogenous ligand at H_1-receptors, possesses an intrinsic activity of 1.0 at the various histamine receptor subtypes (see Table 1) [24]. In contrast, with respect to GTPase stimulation in HL-60 cell membranes, histamine was much less stimulatory than all 2-substituted histamine derivatives studied (see Table 1). Furthermore, HEL cells do not possess histamine receptor subtypes, mediating rises in $[Ca^{2+}]_i$ (see Fig. 2) [26, 27], but compound **9** was quite effective in increasing $[Ca^{2+}]_i$ in these cells via G_i-proteins (see Fig. 2). It is also noteworthy that the stimulatory effects of 2-substituted histamine derivatives on $[Ca^{2+}]_i$ and GTP hydrolysis were evident at concentrations higher than those needed for activation of histamine receptors (see Table 1). Finally, in $1\alpha,25(OH)_2D_3$-differentiated HL-60 cells, histamine increases $[Ca^{2+}]_i$ via H_1-receptors [1], but compounds **7** and **8**, being potent and effective H_1-receptor agonists, failed to induce rises in $[Ca^{2+}]_i$ in these cells (see Table 5). Taken together, all these findings indicate that in HL-60 and HEL cells, 2-substituted histamine derivatives act by a mechanism which is independent of known or novel histamine receptor subtypes.

The fact that 2-substituted histamine derivatives are cationic–amphiphilic substances and that such substances may activate G-proteins by a receptor-independent mechanism [8–10, 18] suggests that this mechanism of action could also account for the effects observed in our present study in HL-60 and HEL cells. This notion is supported by the fact that

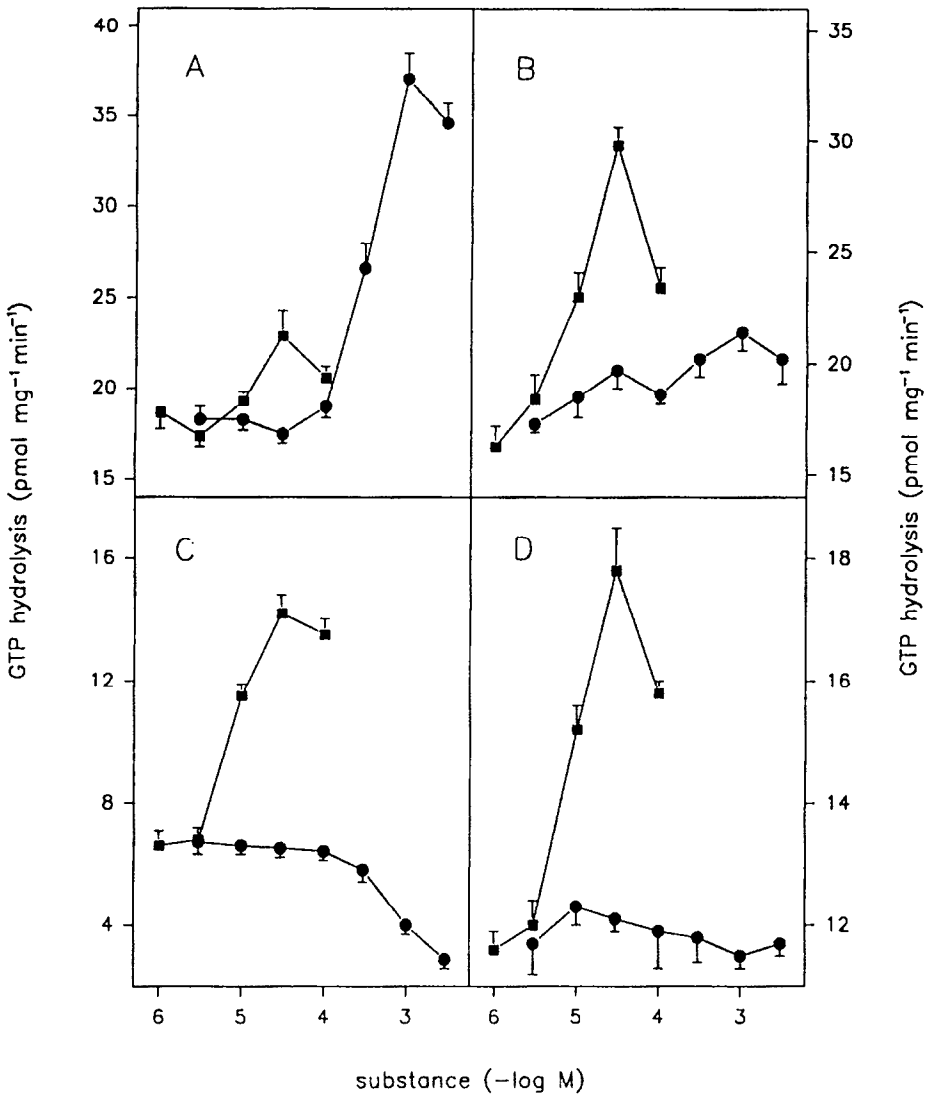


Fig. 4. Effects of MP and compound **9** on GTP hydrolysis in various cell membranes. GTP hydrolysis in cell membranes was determined in the presence of compound **9** (filled circles) and MP (filled squares) at the indicated concentrations. (A) HEL cell membranes; (B) SH-SY5Y cell membranes; (C) RBL 2H3 cell membranes; (D) DDT_1MF-2 cell membranes. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.

there is parallelism of the stimulatory effects of receptor agonists in intact cells and cell membranes [29], whereas 2-substituted histamine derivatives show very different effects in intact HL-60 cells and in cell membranes (see Table 1 and below). However, experiments performed with cell membranes cannot ultimately answer the aforementioned question as the involvement of orphan receptors in the activation process cannot be excluded. In fact, HL-60 cells express such orphan receptors [30]. To compensate for this incompleteness of experiments with cell membranes, we studied the effects of 2-substituted histamine derivatives on the GTPase activity of purified G-proteins. In accordance with our hypothesis, 2-substituted histamine derivatives were found to be effective stimulators of the GTPase of purified G_i/G_o-proteins and of pure G_{i2} (see Table 1 and Fig. 5).

The question arises as to whether in HL-60 cell membranes, G-protein activation induced by 2-substituted histamine derivatives occurs by direct interaction with G-protein α-subunits or indirectly by interaction with NDPK. In a recent study, we have shown that MP may activate G-proteins in HL-60 cell membranes by such a mechanism [14]. Specifically, MP is an effective activator of GTP hydrolysis but not of GTP[γS] binding and photolabeling of G-protein α-subunits with GTP azidoanilide in this system. Most importantly, MP activates GTP formation by NDPK in HL-60 membranes and by purified NDPK. In contrast, compound **8** is effective in activating GTP hydrolysis,

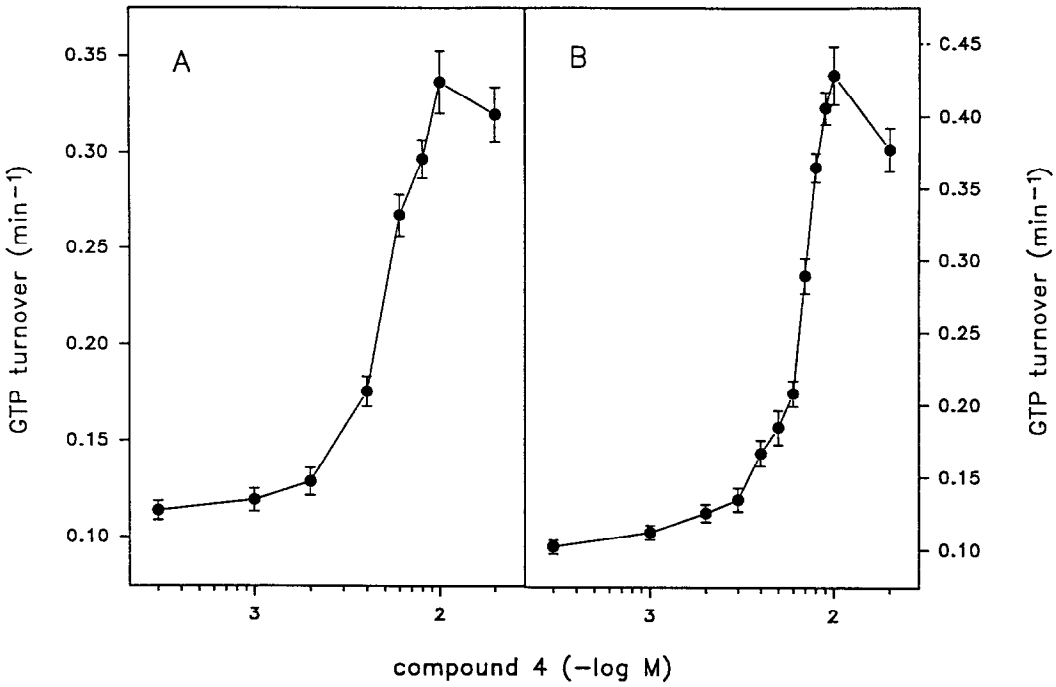


Fig. 5. Concentration–response curves of the stimulatory effect of compound **4** on GTP hydrolysis by purified G_i/G_o-proteins and by pure G_{i2}. GTP hydrolysis by G_i/G_o-proteins (A) and G_{i2} (B) was determined in the presence of compound **4** at the indicated concentrations as described in Materials and Methods. Data shown are the means ± SD of assay quadruplicates. Similar results were obtained in three independent experiments.

GTP[γS] binding and photolabeling of G-protein α-subunits with GTP azidoanilide in HL-60 cell membranes [4]. In addition, 2-substituted histamine derivatives were found not to activate GTP formation in HL-60 cell membranes and by the purified enzyme. Moreover, MP and compound **8** at maximally stimulatory concentrations stimulated GTP hydrolysis in HL-60 cell membranes in a subadditive manner (see Table 4). This subadditivity was increased to synergism in NEM-treated membranes, i.e. in membranes in which the pool of available G_i-proteins had been substantially reduced (see Table 4). All these findings indicate that in HL-60 cell membranes, 2-substituted histamine derivatives and MP interact with different sites and that NDPK does not play a major role in G-protein activation induced by the histamine derivatives.

We observed several differences in the stimulatory effects of 2-substituted histamine derivatives on GTP hydrolysis in cell membranes and by purified G-proteins. For example, the potency of compound **4** to activate GTP hydrolysis by purified G-proteins was considerably lower than that required to activate GTP hydrolysis in HL-60 cell membranes (compare Figs 1 and 5 and Table 1). Differences in the association of G-proteins with phospholipids between the two systems may contribute to the differences in potency of G-protein activators. In addition, the structure–activity relationships of 2-substituted histamine derivatives in HL-60 cell membranes and in the purified system were different (see Table 1). In HL-60 cell membranes, an increase in lipophilicity of substances resulted in an increase of their effectiveness to activate GTP hydrolysis, but with regard to G_i/G_o-proteins, this was not the case (compare **4** with **8** and **4** with **14**). In a membrane system containing G_i- and G_o-proteins (SH-SY5Y cells), compound **9** was only slightly effective in activating GTP hydrolysis, but with respect to purified G_i/G_o-proteins, compound **9** was quite effective (see Table 1 and Fig. 4). Moreover, both in HEL cells and HL-60 cells, 2-substituted histamine derivatives activate G_i-proteins (see Fig. 2) [4], but there are large differences in the effectiveness of substances to activate GTP hydrolysis (compare Table 1 with Fig. 3). In other systems containing G_i-proteins and responsive to MP (RBL 2H3 and DDT_1MF-2 cell membranes), compound **9** did not have a stimulatory effect on GTP hydrolysis (see Fig. 4). Such a cell-type specificity of G-protein activation is not without precedence. Specifically, compound 48/80 is an effective activator of purified G-proteins, but on the level of intact cells, its effects on G-proteins are apparently restricted to mast cells [8, 9].

Receptor-independent G-protein activation by 2-substituted histamine derivatives also shows differences among closely related cell types, i.e. HL-60 promyelocytes and Bt_2cAMP-differentiated HL-60 cells on one side and DMSO- and 1α,25$(OH)_2D_3$-differentiated HL-60 cells and human neutrophils on the other. Specifically, compounds **7** and **8** increased $[Ca^{2+}]_i$ only in the former two cell types but not in the latter three (see Table 5). The lack of stimulatory effect of 2-substituted histamine derivatives on $[Ca^{2+}]_i$ in DMSO-differentiated HL-

60 cells cannot be explained by the absence of G-proteins as MP and compound **7** were effective in increasing GTP hydrolysis in membranes of these cells (see Table 6). Compound **7** may not sufficiently penetrate the plasma membrane in intact DMSO-differentiated HL-60 cells to reach its target, the G-proteins. However, in membrane preparations, these penetration barriers are no longer relevant, allowing compound **7** to activate GTP hydrolysis effectively. By analogy, mast cells from different tissues and species do not show uniform responsiveness to cationic–amphiphilic substances [9]. Mousli *et al.* [9] suggested that the microenvironment of G-proteins may be important for the cell-type selectivity of certain receptor-independent G-protein activators. For example, the lipid environment of G-proteins and their covalent modifications may be different in various cell types and as yet unknown G-protein-associated proteins may contribute to different degrees to the stimulatory effects of cationic–amphiphilic substances. Finally, it should be noted that there are also reports of differentiation-dependent regulation of receptor-mediated signal transduction in HL-60 cells [1, 2, 31]. The molecular basis for these latter differences is also unknown.

A further complication arises from the fact that the structure–activity relationships of 2-substituted histamine derivatives for G_i-protein activation in intact HL-60 cells differ substantially from those in HL-60 cell membranes. Lipophilicity of 2-substituted histamine derivatives does not correlate with their ability to activate G_i-proteins in intact cells, unlike the case in membranes (see Table 1). Interestingly, only compounds with planar ring systems (phenyl rings, compounds **4**, **5** and **7–10**, thienyl rings, compounds **18** and **19**) possess the ability to induce rises in $[Ca^{2+}]_i$, whereas the more bulky non-planar cyclohexyl ring is deleterious in this regard, even in the absence of an alkyl bridge between imidazole and the substituted ring system (compounds **15–17**). In contrast, the presence of a cyclohexyl group is favourable for effective G-protein activation in membranes. The incorporation of an alkyl bridge between imidazole and the phenyl ring (compare compounds **4**, **12** and **14**) or thienyl ring (compare compounds **18** and **19**) renders substances more bulky as well and, again, less stimulatory in intact Bt_2cAMP-differentiated HL-60 cells. Compared to the phenyl ring, the thienyl ring is smaller, and in this case, the incorporation of a methylene bridge results in a much less prominent reduction of the ability of the substance to increase $[Ca^{2+}]_i$ (**4** versus **12** and **18** versus **19**). In contrast, in HL-60 cell membranes, alkyl bridges enhance the stimulatory effects of 2-substituted histamine derivatives (compare compounds **4**, **12** and **14**; **15–17** and **18** with **19**, respectively). Unlike in HL-60 cell membranes, chlorination of the phenyl ring in the *para* position (compound **6**) resulted in a complete loss of ability of the substances to increase $[Ca^{2+}]_i$. Compared to compound **8**, the halogen substituent in the *para* position of the phenyl ring in compound **6** renders the substance more bulky. Taken together, all these findings suggest that the bulkiness of substances prevents them from penetrating the plasma membrane to reach G-proteins. As has been pointed out above, such barriers do not exist in suspended membranes. This rule has, however, an exception: compound **10** is bulkier than compound **9** and is more effective in increasing $[Ca^{2+}]_i$ (see Table 1).

The data concerning the effects of histamine in HL-60 cells merit discussion. In intact Bt_2cAMP-differentiated HL-60 cells, the effects of histamine on $[Ca^{2+}]_i$ are mediated by H_1-receptors, coupling to G_i-proteins and PTX-insensitive G-proteins (see Table 3) [2]. However, in HL-60 cell membranes, histamine showed a stimulatory effect on GTPase which was fully inhibited by PTX but not by histamine receptor antagonists (see Table 3). These data suggest that histamine also possesses the ability to activate G-proteins in a receptor-independent manner, at least in the HL-60 cell system. Interestingly, certain effects of histamine on myeloid cell differentiation are independent of H_1- and H_2-receptors [32]. Additionally, HL-60 cells take up histamine from the extracellular space and certain leukemia cells show an elevated histamine content [32, 33]. Thus, we cannot exclude the possibility that the concentrations of histamine in membranes of intact HL-60 cells are high enough for receptor-independent G-protein activation to take place. In this context, it should be noted that Brandes *et al.* [34] have postulated the existence of an intracellular histamine receptor. This remains to be clarified, inasmuch as this receptor could be a G-protein.

In conclusion, we have shown that histamine and 2-methylhistamine act as H_1-receptor agonists in Bt_2cAMP-differentiated HL-60 cells. Histamine derivatives with bulky and lipophilic substituents at the 2-position activate G_i-proteins in HL-60 and HEL cells by a mechanism which is independent of known histamine receptor subtypes and receptors with as yet unknown ligands. Rather, these substances are receptor-independent G-protein activators. Receptor-independent G-protein activation by 2-substituted histamine derivatives is cell-type specific. The relatively simple structure of 2-substituted histamine derivatives, which can be modified in a logical manner, renders these substances a suitable starting point for the systematic analysis of the structure–activity relationships of receptor-independent G-protein activators. Finally, scientists working with 2-substituted histamine derivatives have to take into consideration the possibility that the otherwise inexplicable effects of these substances are due to receptor-independent G-protein activation. This potential pitfall in the analysis of histamine receptors also applies to other cationic–amphiphilic ligands at these receptors such as arpromidine-derived guanidines and a histamine trifluoromethyl-toluidide derivative [35].

Acknowledgements—The authors are grateful to Mrs E. Glaß, Miss I. Reinsch and Miss R. Bonnet for their expert technical assistance. This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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