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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 from intracellular stores

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Abstract. The results of binding studies suggest the presence of histamine H_1-receptors in human monocytes, but it is not known whether these receptors are functionally active. This prompted us to study the effects of histamine (HA) on cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and superoxide anion (O_2^-) formation in HL-60 cells differentiated towards monocytes with 1α,25-dihydroxycholecalciferol. In HL-60 monocytes, HA increased $[Ca^{2+}]_i$ with a half-maximal effect at 8 μM and a maximum at 30–100 μM. Pertussis toxin (PTX) partially inhibited the stimulatory effects of HA on $[Ca^{2+}]_i$. Betahistine, a weak partial H_1-receptor agonist, also increased $[Ca^{2+}]_i$, whereas H_2- and H_3-receptor agonists were ineffective. H_1- but not H_2- and H_3-receptor antagonists inhibited HA-induced rises in $[Ca^{2+}]_i$. HA-induced rises in $[Ca^{2+}]_i$ were desensitized in a homologous manner and were also inhibited by the activator of protein kinase C, 4β-phorbol 12-myristate 13-acetate. Various protein kinase C inhibitors did not interfere with homologous desensitization. The stimulatory effects of HA on $[Ca^{2+}]_i$ were completely dependent on the presence of extracellular Ca^{2+} and were inhibited by the blocker of non-selective cation (NSC) channels, 1-{β-[3-(4-methoxyphenyl)propoxyl]-4-methoxyphenethyl}-1 H-imidazole hydrochloride (SK & F 96365). HA was much less effective than the chemotactic peptide, N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), to induce rises in $[Ca^{2+}]_i$. Unlike fMLP, HA did not activate O_2^- formation. Our data indicate that HL-60 monocytes possess H_1-receptors coupled to heterotrimeric regulatory guanine nucleotide-binding proteins (G-proteins) of the G_i-family and PTX-insensitive G-proteins which mediate activation of NSC channels without concomitant activation of Ca^{2+} mobilization from intracellular stores, that homologous desensitization of HA-induced Ca^{2+} influx is independent of protein kinase C and that the stimulatory effect of HA on Ca^{2+} influx is too small to result in activation of O_2^- formation.

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Key words: HL-60 monocytes – Histamine H_1-receptors – G-proteins – Pertussis toxin – Non-selective cation channels – Superoxide anion formation

Introduction

Histamine (HA) is an intercellular signal molecule which exerts its effects through H_1-, H_2- and H_3-receptors (Buschauer et al. 1989; Hill 1990). It is well known that human phagocytes, i.e., neutrophils and monocytes, possess H_2-receptors which mediate activation of adenylyl cyclase with subsequent increase in cAMP (Gespach and Abita 1982; Gespach et al. 1982, 1985). The HA-induced increase in cAMP results in inhibition of formyl peptide-induced superoxide anion (O_2^-) formation and in the induction of differentiation (Seligman et al. 1983; Burde et al. 1989, 1990; Nonaka et al. 1992).

Compared to H_2-receptors, little is known about H_1-receptors in human phagocytes. HL-60 cells differentiated towards neutrophils with dibutyryl cAMP possess H_1-receptors coupled to pertussis toxin (PTX)-sensitive heterotrimeric regulatory guanine nucleotide-binding proteins (G-proteins) of the G_i-family and PTX-insensitive G-proteins (Seifert et al. 1992). Occupation with agonist of H_1-receptors in HL-60 neutrophils results in the activation of phospholipase C with subsequent Ca^{2+} mobilization from intracellular stores and Ca^{2+} influx through non-selective cation (NSC) channels (Seifert et al. 1992). The results of binding studies suggest that human monocytes possess H_1-receptors as well, but it is not known whether these receptors are functionally active (Cameron et al. 1986; Driver et al. 1989). This prompted us to study the effects of HA on $[Ca^{2+}]_i$ and O_2^- formation in HL-60 cells differentiated towards monocytes with 1α,25-dihydroxycholecalciferol (1α,25$(OH)_2D_3$) (Ostrem et al. 1987; Hruska et al. 1988). We show here that HL-60 monocytes possess H_1-receptors coupled to G_i-proteins and PTX-insensitive G-proteins which mediate activation of Ca^{2+} influx.

Materials and methods

Materials. Betahistine, dimaprit, impromidine, arpromidine and (R)-α-methylhistamine were gifts from Drs. A. Buschauer and W. Schunack (Institut für Pharmazie, Freie Universität Berlin). Thioperamide was obtained from RBI (Natick, MA, USA). 1α,25(OH)$_2$D$_3$ was a gift from Drs. A. Kaiser and U. Fischer (Hoffman-La Roche, Basel, Switzerland). 1-{β-[3-(4-Methoxyphenyl)propoxyl]-4-methoxyphenethyl}-1 H-imidazole hydrochloride (SK&F 96365) was kindly provided by Dr. J. E. Merritt (SmithKline Beecham, Welwyn, Herts., U.K.). 12-(2-Cyanoethyl)-6,7,12,13-tetrahydro-13-methyl-5-oxo-5 H-indolo[2,3-a]pyrrolo[3,4-c]-carbazole (Gö 6976) and 2-(1 H-indol-3-yl)-3-[1-(3-dimethylaminopropyl)-1 H-indol-3-yl]-maleinimide (Gö 6850) were kindly donated by Dr. C. Schächtele (Gödecke AG, Freiburg/Br., Germany). Fura-2-acetoxymethylester was from Calbiochem (Frankfurt/M., Germany). PTX was from List Biological Laboratories (Campbell, CA, USA). Staurosporine was purchased from Fluka (Buchs, Switzerland). (±)-Chlorpheniramine was from Sigma Chemie (Deisenhofen, Germany). [^{32}P]NAD (800 Ci/mmol) was obtained from Dupont/New England Nuclear (Bad Homburg, Germany). Sources of other materials have been described elsewhere (Rosenthal et al. 1986; Seifert etal. 1989, 1992; Burde et al. 1989, 1990; Wenzel-Seifert and Seifert 1990; Krautwurst et al. 1992).

Cell culture. HL-60 cells 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 °C. To induce monocytic differentiation, HL-60 cells were seeded at 1×10^6 cells/ml and were cultured for 120 h with 10 nM 1α,25(OH)$_2$D$_3$ (Jungblut and Seifert 1990). In some experiments, PTX (1 μg/ml) or carrier (control) were added to the 1α,25(OH)$_2$D$_3$-treated cells 24 h before experiments or membrane preparation.

Membrane preparation. Membranes from 1α,25(OH)$_2$D$_3$-differentiated HL-60 cells were prepared as described (Seifert and Schultz 1987).

PTX-catalyzed ADP-ribosylation of HL-60 membranes. PTX-catalyzed ADP-ribosylation of HL-60 membranes was performed as described (Rudolph et al. 1989). Briefly, reaction mixtures contained 100 μg of membrane protein of PTX- or carrier-treated HL-60 monocytes, 2 μg of activated PTX, 0.3% (w/v) Lubrol PX and 1 μM [^{32}P]NAD (2 μCi/tube) in 25 mM Tris/HCl, pH 7.5. Reactions were conducted for 30 min at 30 °C. Separation of precipitated proteins by SDS PAGE and autoradiography were performed as described (Rosenthal et al. 1986). Bands corresponding to 40/41-kDa proteins were excised from gels, and radioactivity was determined by liquid scintillation counting.

Measurement of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). $[Ca^{2+}]_i$ was determined with the dye, Fura-2-acetoxymethylester, according to the protocol described in detail in Seifert et al. (1992). Briefly, HL-60 monocytes were suspended at 5.0×10^6 cells/ml in a buffer consisting of (mM) 138 NaCl, 6 KCl, 1 $MgSO_4$, 1 Na_2HPO_4, 5 $NaHCO_3$, 5.5 glucose, and 20 HEPES/NaOH, pH 7.4, supplemented with 0.1% (w/v) bovine serum albumin. Cells were incubated for 1 h at 37 °C in the presence of 2 μM Fura-2-acetoxymethylester. Subsequently, cells were diluted with the above buffer to a final concentration of 0.5×10^6 cells/ml and were centrifuged for 10 min at 250×g at room temperature. Cells were suspended at 1.0×10^6 cells/ml in the above buffer and were kept at room temperature until measurement of $[Ca^{2+}]_i$. HL-60 monocytes were used for up to 3 h after dye-loading. Fluorescence of HL-60 monocytes (1.0×10^6 cells in 2 ml) was determined at 37 °C under constant stirring at 10^3 rpm using a Ratio II spectrofluorometer (Aminco, Silver, Spring, MD, USA). The excitation and emission wavelengths were 340 and 500 nm, respectively. Basal $[Ca^{2+}]_i$ in freshly loaded HL-60 monocytes was 107±12 nM (mean±SD, $n = 8$). Unless stated otherwise, experiments were performed in the presence of 1 mM $CaCl_2$.

Assay for O_2^- formation. O_2^- formation was monitored by continuous measurement of ferricytochrome C reduction inhibitable by superoxide dismutase, using an Uvikon 810 dual-beam spectrophotometer (Kontron, Eching, Germany) (Seifert et al. 1989). In brief, reaction mixtures (0.5 ml) contained 2.5×10^6 HL-60 monocytes, 100 μM ferricytochrome C and 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. Reactions were conducted at 37 °C. The absolute amounts of O_2^- generated were calculated.

Data reproducibility. Data shown in Fig. 1 and Tables 1 and 2 are the means±SD of four separate experiments. In Figs. 2–4, representative original tracings are shown. Similar results were obtained in at least three experiments with different preparations of HL-60 cells.

Results

Figure 1 shows the concentration/response curve for the stimulatory effect of HA on $[Ca^{2+}]_i$ in HL-60 monocytes. HA increased $[Ca^{2+}]_i$ with a half-maximal effect at 8 μM and a maximum at 30–100 μM. PTX abolished the stimulatory effect of HA (3 μM) on $[Ca^{2+}]_i$ and diminished those of HA at higher concentrations. The chemotactic peptide, N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), at a maximally effective concentration (1 μM), increased $[Ca^{2+}]_i$ by 923±64 nM (mean±SD, $n = 4$). PTX abolished rises in $[Ca^{2+}]_i$ induced by fMLP (1 μM) (data not shown). In order to answer the question how effectively PTX ADP-ribosylated G_i-protein α-subunits in intact HL-60 monocytes, membranes of PTX- and carrier-treated HL-60 monocytes were subjected to an additional PTX-catalyzed ADP-ribosylation in vitro, using [^{32}P]NAD as substrate. Radioactivity in 40/41-kDa proteins (corresponding to the α-subunits of G_i-proteins) in membranes from PTX-treated and carrier-treated cells amounted to 0±0 and 219±50 counts/min (means±SD, $n = 4$), respectively. These data show that PTX had completely ADP-ribosylated G_i-protein α-subunits in intact cells.

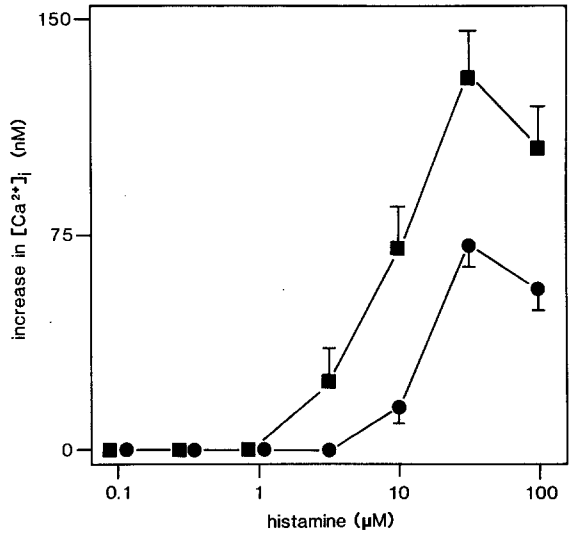

Fig. 1. Concentration/response curves for HA-induced rises in $[CA^{2+}]_i$ in HL-60 monocytes: Effect of PTX. 1α,25(OH)$_2$D$_3$-differentiated HL-60 cells were treated with PTX (1 μg/ml) or carrier (control) for 24 h. Thereafter, cells were harvested, loaded with Fura-2-acetoxymethylester, and the effects of HA at various concentrations on $[Ca^{2+}]_i$ were assessed. ■, control cells; ●, PTX-treated cells. PTX had no effect on basal $[Ca^{2+}]_i$ in HL-60 monocytes

Table 1. Effects of H_1-, H_2- and H_3-receptor agonists on $[Ca^{2+}]_i$ in HL-60 monocytes

Addition	Increase in $[Ca^{2+}]_i$ (nM)
Histamine (100 μM)	112±13
Betahistine (100 μM)	38±15
Dimaprit (100 μM)	0
Impromidine (100 μM)	0
Apromidine (100 μM)	0
(*R*)-α-Methylhistamine (100 μM)	0

HL-60 cells were harvested, loaded with Fura-2-acetoxymethylester, and the effects of various H_1-, H_2- and H_3-receptor agonists on $[Ca^{2+}]_i$ were assessed

The effects of H_1-, H_2- and H_3-receptor agonists on $[Ca^{2+}]_i$ in HL-60 monocytes were studied (Table 1). The weak partial H_1-receptor agonist, betathistine (100 μM) (Zingel and Schunack 1993), increased $[Ca^{2+}]_i$ with an effectiveness amounting to 34% of that of HA (100 μM). By contrast, the H_2-receptor agonists, dimaprit, impromidine and arpromidine (Buschauer 1989; Buschauer et al. 1989; Hill 1990), and the H_3-receptor agonist, (R)-α-methylhistamine (Buschauer et al. 1989; Hill 1990) did not induce rises in $[Ca^{2+}]_i$ in HL-60 monocytes.

Table 2 shows the effects of H_1-, H_2- and H_3-receptor antagonists on HA-induced rises in $[Ca^{2+}]_i$ in HL-60 monocytes. The H_1-receptor antagonists, diphenhydramine, (±)-chlorpheniramine and clemastine (10 μM each) (Buschauer et al. 1989; Hill 1990), inhibited rises in $[Ca^{2+}]_i$ induced by HA (10 μM). By contrast, the H_2-receptor antagonists, cimetidine and famotidine (10 μM each) (Schunack 1987; Buschauer et al. 1989; Hill 1990), and the H_3-receptor antagonist, thioperamide (10 μM) (Hill 1990), had no inhibitory effect on HA-induced rises in $[Ca^{2+}]_i$.

Desensitization of HA-induced rises in $[Ca^{2+}]_i$ was studied according to the procedure described by Schwaner et al. (1992), i.e., agonist was re-added to cells 3 min after its first addition. HA (100 μM) caused a rapid increase in $[Ca^{2+}]_i$ in HL-60 monocytes which returned to basal values within less than 2 min (Fig. 2A). Re-addition of HA (100 μM or 1 mM) did not result in another rise in $[Ca^{2+}]_i$ (see Fig. 2A). Pretreatment of HL-60 monocytes with the inhibitor of various protein kinases including protein kinase C, staurosporine (1 μM) (Tamaoki et al. 1986), the inhibitor of Ca^{2+}-dependent protein kinase C isoenzymes, Gö 6976 (0.1 and 1 μM) (Martiny-Baron et al. 1993), or with the inhibitor of Ca^{2+}-dependent and Ca^{2+}-independent protein kinase C isoenzymes, Gö 6850 (0.1 and 1 μM) (Martiny-Baron et al. 1993), did not affect the stimulatory effect of HA on $[Ca^{2+}]_i$ (first addition of stimulus) and did also not result in appearance of a stimulatory effect of HA on $[Ca^{2+}]_i$ (second addition of stimulus) (data not shown).

The activator of protein kinase C, 4β-phorbol 12-myristate 13-acetate (PMA), did not induce a rise in $[Ca^{2+}]_i$ but abolished the stimulatory effect of HA on $[Ca^{2+}]_i$ (see Fig. 2B, C). By contrast, a phorbol ester which does not activate protein kinase C, 4α-phorbol-12,13,-didecanoate (PDD), did not affect HA-induced rises in $[Ca^{2+}]_i$ (see Fig. 2D).

The effects of extracellular Ca^{2+} and of the NSC channel blocker, SK&F 96365 (Merritt et al. 1990; Krautwurst et al. 1992), on fMLP- and HA-induced rises in $[Ca^{2+}]_i$ in HL-60 monocytes are shown in Fig. 3. The fMLP-induced incrase in $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+} was much greater and more sustained than the one induced by HA. In the absence of extracellu-

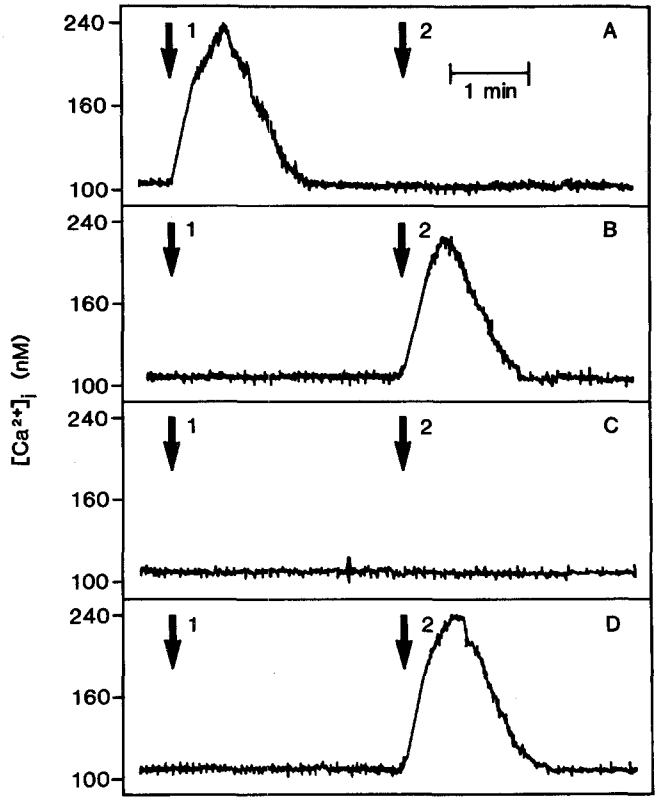

Fig. 2A–D. Desensitization of HA-induced rises in $[Ca^{2+}]_i$ in HL-60 monocytes. HL-60 cells were harvested, loaded with Fura-2-acetoxymethylester, and the effects of HA on $[Ca^{2+}]_i$ under various experimental conditions were assessed. **A** *1*, addition of HA (100 μM); *2*, addition of HA (100 μM or 1 mM). **B** *1*, addition of solvent (control); *2*, addition of HA (100 μM). **C** *1*, addition of PMA (100 ng/ml); *2*, addition of HA (100 μM). **D** *1*, addition of PDD (100 ng/ml); *2*, addition of HA (100 μM). Original fluorescence tracings are shown

Table 2. Effects of H_1-, H_2- and H_3-receptor antagonists on HA-induced rises in $[Ca^{2+}]_i$ in HL-60 monocytes

Addition	Increase in $[Ca^{2+}]_i$ (nM)
Solvent (control)	69±12
Diphenhydramine (10 μM)	6±5
(±)-Chlorpheniramine (10 μM)	7±3
Clemastine (10 μM)	9±7
Cimetidine (10 μM)	70±5
Famotidine (10 μM)	75±13
Thioperamide (10 μM)	68±15

HL-60 cells were harvested, loaded with Fura-2-acetoxymethylester, and the effects of HA (10 μM) on $[CA^{2+}]_i$ were assesed in the absence or presence of various H_1-, H_2- or H_3-receptor antagonists. H_1-, H_2- or H_3-receptor antagonists were added to cells 3 min before HA. H_1-, H_2- and H_3-receptor antagonists by themselves had no effect on $[Ca^{2+}]_i$

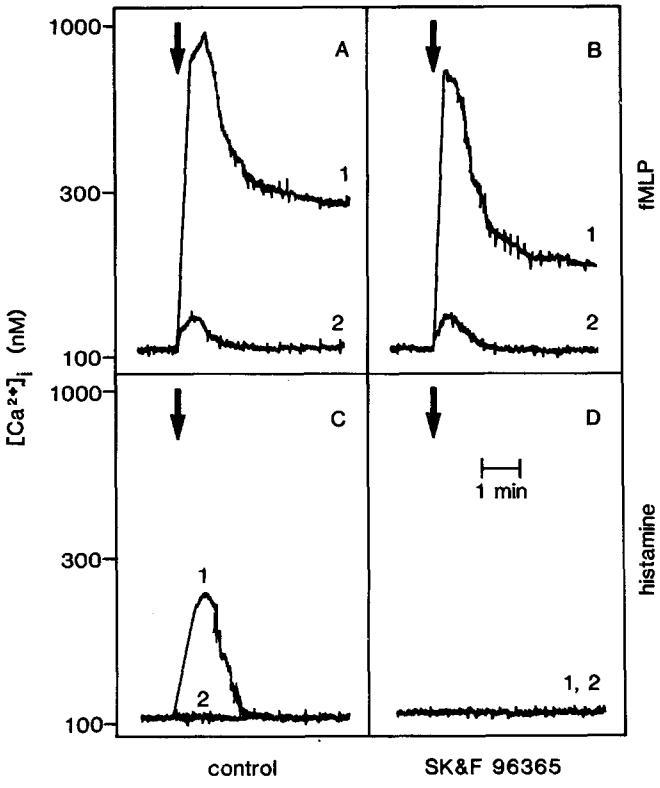

Fig. 3A–D. Effects of extracellular Ca^{2+} and of SK&F 96365 on fMLP- and HA-induced rises in $[Ca^{2+}]_i$ in HL-60 monocytes. HL-60 cells were harvested, loaded with Fura-2-acetoxymethylester, and the effects of fMLP (1 μM, **A**, **B**) and HA (100 μM, **C**, **D**) on $[Ca^{2+}]_i$ were assessed. *Arrows* indicate the addition of stimuli. Three min before the addition of stimuli, solvent (control, **A**, **C**) or SK&F 96365 (30 μM, **B**, **D**) were added to cells. Trace *1*, presence of $CaCl_2$ (1 mM); trace *2*, presence of EGTA (1 mM) without added $CaCl_2$. Superimposed original fluorescence tracings are shown

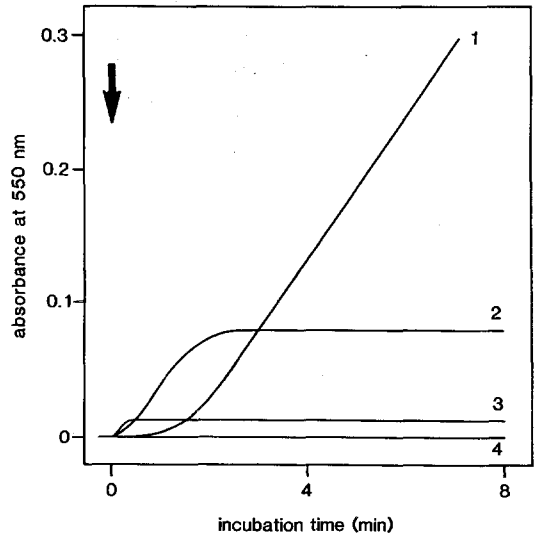

Fig. 4. Time courses of O^{2-} formation in HL-60 monocytes. HL-60 cells were harvested and O_2^- formation was assessed under various experimental conditions. The *arrow* indicates the addition of stimuli. In some experiments, cytochalasin B (1 μg/ml) was added to cells 3 min before the addition of stimuli. Trace *1*, PMA (100 ng/ml); trace *2*, fMLP (1 μM) with cytochalasin B; trace *3*, fMLP (1 μM) without cytochalasin B; trace *4*, HA (100 μM) with or without cytochalasin B

lar Ca^{2+}, fMLP induced only a very small increase in $[Ca^{2+}]_i$. SK&F 96365 (30 μM) partially inhibited the stimulatory effect of fMLP on $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+} (39±15% inhibition of peak $[Ca^{2+}]_i$ values, mean±SD, $n = 5$, $P<0.05$ as assessed by the Wilcoxon test). SK&F 96365 did not inhibit the stimulatory effect of fMLP on $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. The HA-induced rise in $[Ca^{2+}]_i$ was completely dependent on the presence of extracellular Ca^{2+}. SK&F 96365 (30 μM) abolished the stimulatory effect of HA on $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+}.

Finally, we studied activation of the O_2^--forming NADPH oxidase in HL-60 monocytes (Fig. 4). After a lag time of about 1 min, PMA effectively activated O_2^- formation (14.8±1.5 nmol of O_2^-/min/10^6 cells, mean±SD, $n = 3$). By contrast to PMA, fMLP at a maximally stimulatory concentration (1 μM) induced only a small and very short-lasting formation of O_2^- (0.12±0.04 nmol of $O_2^-/10^6$ cells, mean±SD, $n = 3$). Cytochalasin B potentiated this O_2^- formation several-fold (0.83±0.15 nmol of $O_2^-/10^6$ cells, mean±SD, $n = 3$). HA (100 μM) did not activate O_2^- formation, regardless or whether cytochalasin B was present or not.

Discussion

The results of binding studies suggest that human monocytes possess H_1-receptors (Cameron et al. 1986; Driver et al. 1989). H_1-receptors mediate activation of phospholipase C with subsequent Ca^{2+} mobilization from intracellular stores (Hill 1990). These findings prompted us to study the effects of HA on $[Ca^{2+}]_i$ and O_2^- formation in HL-60 monocytes. HA, in a concentration-dependent manner, increased $[Ca^{2+}]_i$ in HL-60 monocytes, and the effects of HA were inhibited by H_1-receptor antagonists but not by H_2- and H_3-receptor antagonists (see Fig. 1, Table 2). In addition, a partial H_1-receptor agonist (betahistine) had a small stimulatory effect on $[Ca^{2+}]_i$, whereas various H_2-receptor agonists and an H_3-receptor agonist failed to increase $[Ca^{2+}]_i$ (see Table 1). These data show that HL-60 monocytes possess functionally active H_1-receptors which mediate increases in $[Ca^{2+}]_i$.

The finding that the cDNAs of H_1-receptors from various species possess several potential phosphorylation sites for protein kinases including protein kinase C (Yamashita et al. 1991; Fujimoto et al. 1993; Horio et al. 1993) prompted us to study desensitization of HA-induced rises in $[Ca^{2+}]_i$ in HL-60 monocytes. Repeated addition of HA to HL-60 monocytes did not result in another rise in $[Ca^{2+}]_i$ (see Fig. 2A). These findings show that H_1-receptors in phagocytes undergo homologous desensitization as is the case for formyl peptide receptors (Seifert et al. 1989; Didsbury et al. 1991). Protein kinase C is apparently not involved in homologous desensitization as various protein kinase C inhibitors failed to prevent this process. Thus, by analogy to other G-protein-coupled receptors (Lefkowitz 1993), a specific receptor kinase may mediate homologous desensitization of HA-induced rises in $[Ca^{2+}]_i$ in HL-60 monocytes. How-

ever, heterologous desensitization (Didsbury et al. 1991) of HA-induced rises in $[Ca^{2+}]_i$ in HL-60 monocytes C may be mediated through protein kinase C. This notion is supported by the finding that the protein kinase C-activating phorbol ester, PMA, inhibited HA-induced rises in $[Ca^{2+}]_i$, whereas an inactive phorbol ester, PDD, was ineffective (see Fig. 2B–D).

The sequence analysis of the cDNAs of H_1-receptors shows that they possess seven putative membrane-spanning domains, i.e., they belong to the superfamily of G-protein-coupled receptors (Yamashita et al. 1991; Fujimoto et al. 1993; Horio et al. 1993). In order to characterize the G-proteins coupled to H_1-receptors in HL-60 monocytes, the effect of PTX on HA-induced rises in $[Ca^{2+}]_i$ was studied. As is the case for neutrophils, G_{i2} is the most abundant PTX-sensitive G-protein in monocytes (Murphy et al. 1987; Pollock et al. 1990). Under the conditions employed, PTX completley ADP-ribosylated G_i-protein α-subunits in intact HL-60 monocytes. PTX abolished rises in $[Ca^{2+}]_i$ induced by fMLP at a maximally stimulatory concentration in HL-60 monocytes, indicating that they are fully mediated through G_i-proteins. By comparison, HA-induced rises in $[Ca^{2+}]_i$ were only partially inhibited by PTX. Thus, in addition to G_i-proteins, PTX-insensitive G-proteins are involed in the signal transduction pathway activated by HA (see Fig. 1). The identify of the PTX-insensitive G-protein(s) in presently unknown, but a candidate in this regard is G_{16} which is expressed exclusively in white blood cells (Amatruda et al. 1991). Alternatively, the more widely distributed PTX-insensitive G-proteins, G_{12} and G_{13}, may be involed in the signal transduction pathway (Strathmann and Simon 1991).

Usually, H_1-receptor-mediated rises in $[Ca^{2+}]_i$ are due to mobilization of Ca^{2+} from intracellular stores as a consequence of phospholipase C activation (Hill 1990). In addition, agonist-occupied H_1-receptors may cause activation of Ca^{2+} influx from the extracellular space (Hill 1990). In HL-60 monocytes, HA-induced rises in $[Ca^{2+}]_i$ are exclusively due to Ca^{2+} influx without concomitant Ca^{2+} mobilization as its stimulatory effects were completely dependent on the presence of extracellular Ca^{2+} (see Fig. 3). By analogy to HL-60 neutrophils, the effects of HA on Ca^{2+} influx in HL-60 moncytes were inhibited by SK & F 96365, indicating that Ca^{2+} influx is mediated through NSC channels (see Fig. 3) (Seifert et al. 1992).

The precise mechanisms by which intercellular signal molecules activate NSC channels in general and in human phagocytes in particular are unknown, but one current hypothesis states that Ca^{2+} mobilization from intracellular stores is a prerequisite for activation of Ca^{2+} influx (Demaurex et al. 1992; Alonso-Torre et al. 1993; Randriamampita and Tsien 1993; Clapham 1993). The data obtained with HA in HL-60 monocytes do not support this hypothesis as Ca^{2+} influx occurred without concomitant Ca^{2+} mobilization (see Fig. 3). In addition, fMLP only very weakly activated Ca^{2+} mobilization in HL-60 monocytes but was quite effective with respect to Ca^{2+} influx (see Fig. 3). Similar to the results obtained with HA in $1\alpha,25(OH)_2D_3$-differentiated HL-60 cells, certain cytokines stimulate Ca^{2+} influx through NSC channels in human monocytes without activating Ca^{2+} mobilization (see Fig. 3) (Sozzani et al. 1993). Moreover, complement component C3a, unlike complement component C5a, induces only Ca^{2+} influx but not Ca^{2+} mobilization in human neutrophils (Norgauer et al. 1993). Furthermore, complement C5a-induced Ca^{2+} influx in dibutyryl cAMP-differentiated U937-cells apparently does not depend on prior emptying of intracellular Ca^{2+} stores (Monk and Partridge 1993). Dissociations between receptor agonist-mediated Ca^{2+} mobilization and Ca^{2+} influx were also reported for rat pancreatic acinar cells (Dawra et al. 1993) and rat thyroid cells (Aloj et al. 1993). All these findings support our recent suggestion that NSC channels in human phagocytes are under a more direct control of G-proteins (Krautwurst et al. 1992).

Stimulation of Ca^{2+} influx through NSC channels by receptor agonists in HL-60 neutrophils is involved in the activation of O_2^- formation (Krautwurst et al. 1992). By analogy, in HL-60 monocytes, fMLP stimulated Ca^{2+} influx and O_2^- formation (see Figs. 3, 4). HA was much less effective than fMLP to stimulate Ca^{2+} influx and did not activate O_2^- formation (see Figs. 3, 4). These data indicate that the HA-induced Ca^{2+} influx in HL-60 monocytes was too small to result in activation of O_2^- formation. Possibly, the HA-induced rises in $[Ca^{2+}]_i$ in HL-60 monocytes play a part in the regulation of gene expression. This assumption is supported by the finding that rises in $[Ca^{2+}]_i$ (induced by ionomycin) with similar magnitude and duration as those induced by HA in HL-60 monocytes are sufficent to modulate the expression of various genes in undifferentiated HL-60 cells (Werlen et al. 1993).

There are both similarities and differences in the effects of HA and fMLP in dibutyryl cAMP-differentiated and $1\alpha,25(OH)_2D_3$-differentiated HL-60 cells. In both cell types, H_1-receptors are coupled to G_i-proteins and PTX-insensitive G-proteins (see Fig. 1) (Seifert et al. 1992). In either cell type, HA-induced rises in $[Ca^{2+}]_i$ are desensitized in homologous and heterologous manners (see Fig. 2) (Seifert et al. 1992; R. Seifert, unpublished results). In addition, HA does not activate O_2^- formation in HL-60 neutrophils and HL-60 monocytes (see Fig. 4) (Seifert et al. 1992). Moreover, the peak $[Ca^{2+}]_i$ values induced by fMLP at maximally effective concentrations in dibutyryl cAMP-differentiated and $1\alpha,25(OH)_2D_3$-differentiated HL-60 cells are comparable (see Fig. 3) (Wenzel-Seifert and Seifert 1990; Seifert et al. 1992).

With respect to differences, in dibutyryl cAMP-differentiated HL-60 cells, the effectiveness of HA to increase $[Ca^{2+}]_i$ is greater than in HL-60 monocytes, and the duration of the HA-induced rises in $[Ca^{2+}]_i$ in the latter cells is shorter than in the former ones (see Figs. 2, 3) (Seifert et al. 1992). In addition, HA activates both Ca^{2+} mobilization and Ca^{2+} influx in dibutyryl cAMP-differentiated HL-60 cells, whereas in $1\alpha,25(OH)_2D_3$-differentiated HL-60 cells, only Ca^{2+} influx is activated (see Fig. 3) (Seifert et al. 1992). Moreover, in HL-60 monocytes, fMLP increases $[Ca^{2+}]_i$ exclusively through G_i-proteins, whereas in dibutyryl cAMP-differentiated HL-60 cells, fMLP-induced rises in $[Ca^{2+}]_i$ are partially

PTX-insensitive (Wenzel-Seifert and Seifert 1990; Seifert et al. 1992). Furthermore, in HL-60 monocytes, fMLP is a much less effective activator of O_2^- formation than in HL-60 neutrophils (see Fig. 4) (Seifert et al. 1989, 1992; Krautwurst et al. 1992). This difference is not due to the lack of a functionally active NADPH oxidase as PMA is an effective activator of O_2^- formation in HL-60 monocytes (see Fig. 4). Intriguingly, fMLP-induced Ca^{2+} influx in HL-60 monocytes ceases more rapidly than in HL-60 neutrophils (see Fig. 3) (Seifert et al. 1992; Krautwurst et al. 1992). Thus, the lower effectiveness of fMLP to activate NSC channels in HL-60 monocytes compared to HL-60 neutrophils could account, at least in part, for the differences in effectiveness among these cell types to activate NADPH oxidase. Taken together, the above discussed findings suggest that there are differences in the interaction of H_1-receptors and formyl peptide receptors with G-proteins and/or of G-proteins with effector systems between dibutyryl cAMP-differentiated and $1\alpha,25(OH)_2D_3$-differentiated HL-60 cells. Interestingly, differences in the interactions for formyl peptide receptors with G_i-proteins have also been observed between dibutyryl cAMP-differentiated and dimethyl sulfoxide-differentiated HL-60 cells (Tohkin et al. 1991). Dimethyl sulfoxide-differentiated HL-60 cells possess neutrophil-like properties as well (Seifert and Schultz 1987; Seifert al. 1989).

In conclusion, HL-60 monocytes posses functional H_1-receptors coupled to G_i-proteins and yet unknown PTX-insensitive G-proteins. H_1-receptors mediate activation of NSC channels without concomitant Ca^{2+} mobilization from intracellular stores. The stimulatory effects of HA on NSC channels are too small to result in activation of O_2^- formation but may play a role in the regulation of gene expression.

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