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Histamine Modulates the Expression of c-*fos* through Cyclic AMP Production via the H_2 Receptor in the Human Promonocytic Cell Line U937

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SUMMARY

We examined the effects of histamine and its agonists on the expression of the c-*fos* and c-*myc* proto-oncogenes at the transcriptional and translational levels in the human promonocytic U937 cell line. Histamine transiently increased cAMP and c-*fos* expression through H_2 receptors. Dibutyryl cAMP also increased c-fos mRNA and protein, and levels remained elevated even after 12 hr of treatment. Dose-dependence studies using histamine and dimaprit showed that the EC_{50} values for cAMP production and c-*fos* increase were similar, suggesting that cAMP might be involved in c-*fos* induction via H_2 receptors. Furthermore, studies carried out using H7, a protein kinase A/protein kinase C inhibitor, blocked c-*fos* induction, whereas no effect was observed with bisindolylmaleimide, a specific protein kinase C inhibitor. No modification of c-*myc* expression could be detected on treatment with histamine or its analogues. Nevertheless, dibutyryl cAMP induced a down-regulation of the levels of this proto-oncogene. In addition, dibutyryl cAMP inhibited cell growth in a dose-dependent manner, whereas histamine failed to affect proliferation and differentiation of U937 cells. Cells pretreated with dimaprit showed a decrease in the cAMP response to subsequent addition of H_2 agonists, whereas the cAMP response to prostaglandin E_2 remained unaltered. This homologous mechanism of H_2 receptor desensitization was time dependent. These results indicate that histamine activates several mechanisms involved in the induction of differentiation, such as cAMP and c-*fos* production, but fails to promote differentiation of U937 cells, apparently due to the rapid desensitization of H_2 receptors.

Histamine is an intercellular signal molecule that exerts its effect through H_1, H_2, and H_3 receptors (1). It is well known that human phagocytes possess H_2 receptors, which mediate activation of adenylyl cyclase with subsequent increases in cAMP and PKA activity (2). This in turn modulates different cellular processes such as proliferation and differentiation. Recently, other cAMP-independent pathways have been described for H_2 receptors, including regulation of the breakdown of phosphoinositides (3, 4), of intracellular Ca^{2+} levels (5, 6), and of phospholipase A_2 activity (7). The presence of H_1 and H_2 histamine receptors in U937 cells derived from the monocyte/macrophage lineage (8) has been reported (9, 10). H_2 receptor activation produces an increase in cAMP formation (9, 10), whereas the H_1 receptor seems to be coupled to enhancement of phosphoinositide turnover (9).

Certain subsets of early response genes code for transcription factors involved in nuclear signal transduction. For example, the c-*fos* gene is induced as an immediate early event by serum, growth factors, and phorbol ester (11). The Fos and Jun proteins are the components of the activator protein-1 transcription factor that binds to the consensus sequence TGA(C/G)TCA (TRE) and regulates the transcription of several genes (12). Although Jun is capable of binding to this sequence as a homodimer, the formation of the Jun/Fos heterodimer greatly enhances the ability of these proteins to stimulate transcription from activator protein-1 enhancer sequences (13). Another early response gene, c-*myc*, has long been implicated in the control of normal cell growth, whereas its deregulation is related to the development of neoplasia (14). Myc protein is a short-lived nuclear phosphoprotein that exhibits a dimerization domain structurally related to those described in a number of transcription factors (15).

Different agents such as PMA (16), interferon-γ (17), vita-

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ABBREVIATIONS: PMA, phorbol-12-myristate-13-acetate; PGE_2, prostaglandin E_2; dbcAMP, dibutyryl cAMP; IBMX, isobutylmethylxantine; H7, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine; SDS, sodium dodecyl sulfate; PKA, protein kinase A; PKC, protein kinase C; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; BM, bisindolylmaleimide; NBT, nitro blue tetrazolium.

min D_3 (18), and tumor necrosis factor-α (19) can induce cellular differentiation in the U937 cell line. A decrease in c-*myc* and c-*myb* mRNA levels and an increase in c-*fos* and c-*jun* transcripts have been reported during this event (20, 21).

The aim of the current study was to investigate the effects of histamine and its agonists on the modulation of the c-*fos* and c-*myc* proto-oncogenes and their implication in U937 promonocytic cell differentiation. We demonstrate that histamine and H_2, but not H_1, agonists induce a transient increase in c-*fos* mRNA and protein levels through cAMP production without affecting cell differentiation or proliferation. In addition, these agents do not modulate c-*myc* expression.

Materials and Methods

Chemicals. Histamine dihydrochloride, PGE_2, IBMX, cAMP, H7, famotidine, pyrilamine, dbcAMP, PMA, and rhC5a were purchased from Sigma Chemical (St. Louis, MO). BM (Calbiochem, San Diego, CA), dimaprit (22), and 2-(3-trifluormethylphenyl)histamine (23) were kindly provided by Dr. W. Schunack (Freie Universität Berlin, Berlin, Germany) and Dr. A. Buschauer (Regensburg Universität, Regensburg, Germany). Random primer DNA labeling kit was from BioRad (Hercules, CA). [^{3}H]cAMP and [^{32}P]dCTP were purchased from DuPont-New England Nuclear (Boston, MA). All other chemicals were of analytical grade.

Cell culture. The U937 cell line (American Type Culture Collection, Rockville, MD) was cultured in suspension at 37° in RPMI 1640 medium supplemented with 10% fetal calf serum and 50 μg/ml gentamicin in a 5% CO_2 humidified incubator. Cells were routinely passed every 3 days and seeded at a density of 2.5×10^5 cells/ml.

Northern blots. The levels of c-*fos* and c-*myc* expression under various conditions were examined by Northern blot analysis. Total RNA was isolated as described previously (24), and 20-μg aliquots were electrophoresed in 1% agarose/0.8 M formaldehyde gels, transferred to ZetaProbe membranes (BioRad), and hybridized overnight at 45° in 0.125 M sodium chloride, 0.25 M sodium phosphate, pH 7.2, 5% SDS, 10% polyethylene glycol, 50% formamide, and $>10^6$ cpm/ml random primer ^{32}P-labeled probe. Filters were washed and exposed to AGFA Curix RP1 films. Quantification of films was performed with an LKB scanner (Uppsala, Sweden). Results of specific mRNA hybridization were standardized to the "housekeeping" gene GAPDH and expressed as the ratio of specific mRNA to GAPDH mRNA.

The probes used were (i) 1.8-kb *Xho*I/*Eco*RI fragment of human c-fos cDNA cloned in pBK28 plasmid (generously donated by Dr. I. Verma, Salk Institute, San Diego, CA), (ii) 1.4-kb *Cla*I/*Eco*RI fragment containing human c-*myc* third exon cloned in pHSR1 (American Type Culture Collection, Rockville, MD), and (iii) 1.2-kb *Pst*I fragment of GAPDH cDNA (kindly provided by Dr. A. Kornblihtt, INGEBI-Bs.As., Argentina).

Western blots. Treated and control cells (3×10^6) were lysed in 300 μl of 50 mM Tris·HCl, pH 6.8, 2% SDS, 100 mM 2-mercaptoethanol, 10% glycerol, and 0.05% bromphenol blue and sonicated to shear DNA. Samples were then boiled for 5 min, and 20-μl samples were electrophoresed in 12% SDS-polyacrylamide gels and transferred to nitrocellulose paper. The residual binding sites were blocked with 5% nonfat dried milk in TBST (20 mM Tris·HCl, pH 7.6, 137 mM NaCl, 0.05% Tween-20), and membranes were incubated with 2 μg/ml concentration of the specific primary antibody in 3% nonfat dried milk in TBST. Rabbit sera against Myc, Jun, and Fos proteins were purchased from Santa Cruz Biochemicals (Santa Cruz, CA). All subsequent washes were performed in TBST. Reactivity was developed using an anti-rabbit polyclonal antibody linked to horseradish peroxidase and enhanced chemiluminescence reagents according to the manufacturer's instructions (Amersham International, Buckinghamshire, UK).

cAMP determination. Cells were stimulated with different concentrations of histamine, H_2 agonist (dimaprit), or H_1 agonist (2-(3-trifluormethylphenyl) histamine) in Hanks' balanced salt solution containing 1 mM IBMX. Reaction was terminated by 3-min centrifugation at $3000 \times g$ followed by the addition of ethanol. Supernatants were centrifuged 10 min at $3000 \times g$, and the ethanol phase was dried and resuspended in 50 mM Tris·HCl buffer, pH 7.4. The cAMP content was determined by competition with [^{3}H]cAMP for PKA, as described previously (9).

Desensitization experiments. Pretreatment of cells with dimaprit was performed in Hanks' solution at 37° in a humidified atmosphere containing 5% CO_2. Cells were exposed to 10 μM dimaprit (maximal response) for periods ranging from 1 min to 3 hr in the absence of IBMX, after which cells were washed and resuspended at a density of 10^6 cells/ml in Hanks' solution containing 1 mM IBMX and exposed for 9 min to dimaprit to determine whether the histamine H_2 receptors could still generate a cAMP response.

Cell proliferation. Cells were seeded at 2×10^5 cells/ml and incubated for diverse period of times (maximum, 6 days) with different doses of dimaprit, 2-(3-trifluormethylphenyl)histamine, dbcAMP, and forskolin. Cells were harvested, and their numbers were estimated using an hemocytometer chamber.

Cell differentiation. Control and U937 cells treated for 72 hr with 10 μM dimaprit or 0.4 mM dbcAMP were incubated 15 min at 37° with murine anti-CD14, anti-CD11b (25) antibodies, or the isotypic control. Cells were immunolabeled by the addition of a goat anti-murine IgG/fluorescein isothiocyanate conjugate. All of these reagents were purchased from DAKO (Carpinteria, CA). Labeled cells were analyzed in a FACStar plus flow cytometer (Becton Dickinson, San Jose, CA).

Chemotaxis assay. *In vitro* locomotion of control and treated U937 cells was assayed according to the micropore filter technique. Briefly, 10^5 cells in 0.5 ml of RPMI 1640 supplemented with 0.5% human serum albumin were seeded onto the top compartment of the chemotactic chamber placed in a 24-well culture plate. The top and bottom compartments were separated by a PVP-free polycarbonate filter with a pore size of 5 μm. The bottom compartment was filled with 0.6 ml of control media alone or plus 5 nM rhC5a (26). Chambers were incubated for 1–3 hr at 37° in a 5% CO_2 atmosphere. Migrating cells were collected and counted by flow cytometry (Cytoron; Ortho Diagnosis, Raritan, NJ).

NBT reduction. Treated and control cells were mixed with equal volumes of 0.2% NBT and 200 nM PMA in phosphate-buffered saline. After 1 hr of incubation at 37°, the proportion of cells containing intracellular formazan deposits was determined on May-Grunwald-Giemsa-stained cytospin slide preparations.

Data analysis. EC_{50} values and doses required to reach maximal response were obtained by analyzing the concentration-response curves (see Figs. 2 and 6). Statistical analysis was carried out with Student's *t* test or one-way analysis of variance. A Bonferroni *post hoc* test for multiple pairwise comparisons was performed when significant differences were detected. Values of $p < 0.05$ were considered to indicate statistically significant differences.

Results

Time-dependent induction of c-*fos* expression by histamine and H_2 agonist. U937 cells treated with 10 μM histamine or H_2 agonist showed a remarkable transient increase in c-*fos* levels after 30 min (Fig. 1, *top*). Quantitative analysis of this assay clearly showed maximal levels of c-*fos* mRNA after 60 min of treatment. These mRNA levels consequently diminished, reaching values close to the basal level at 4 hr of treatment (Fig. 1, *bottom*). Identical experiments carried out in the presence of 10 μM H_1 agonist showed no effects on the levels of c-*fos* mRNA. The levels of the control

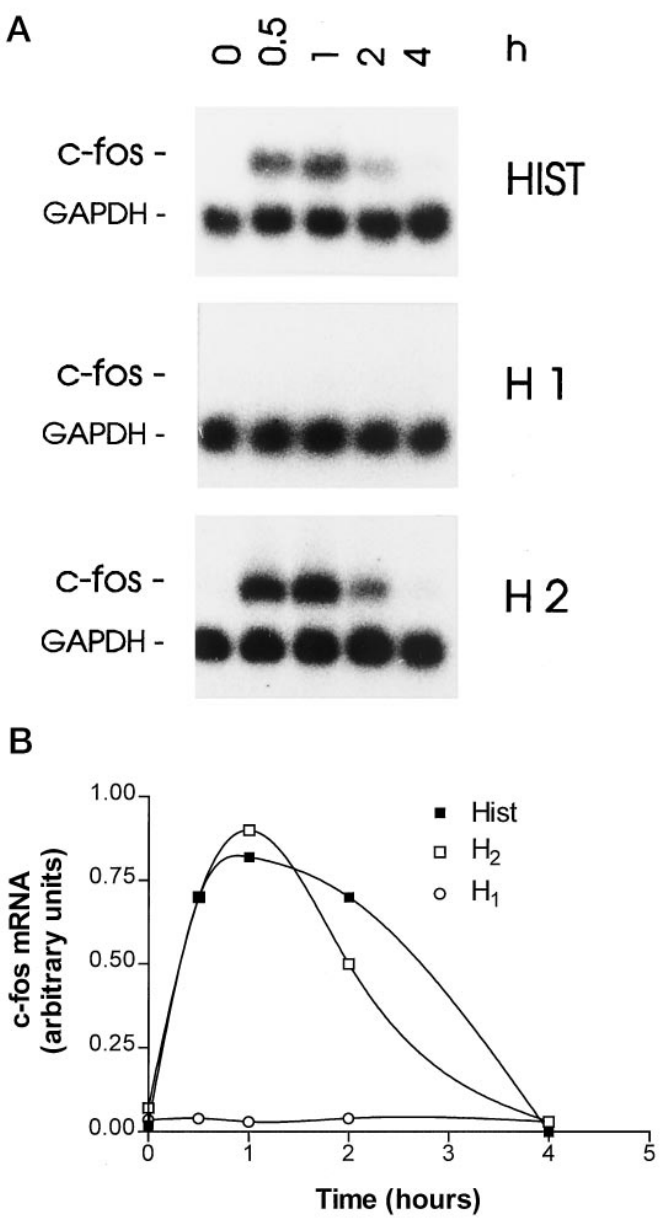

Fig. 1. Time-dependent effects of histamine, H_1 agonist, and H_2 agonist on c-*fos* mRNA levels. U937 cells were cultured in the presence of histamine (*HIST*), 2-(3-trifluormethylphenyl)histamine (*H1*), or dimaprit (*H2*) (10 μM each) for the indicated periods of time. Total cellular RNA (20 μg) was hybridized to ^{32}P-labeled c-*fos* and GAPDH probes. A, autoradiograms. B, quantification of autoradiograms. Arbitrary units represent the ratio of c-*fos* mRNA to GAPDH mRNA. Data are representative of at least three independent experiments.

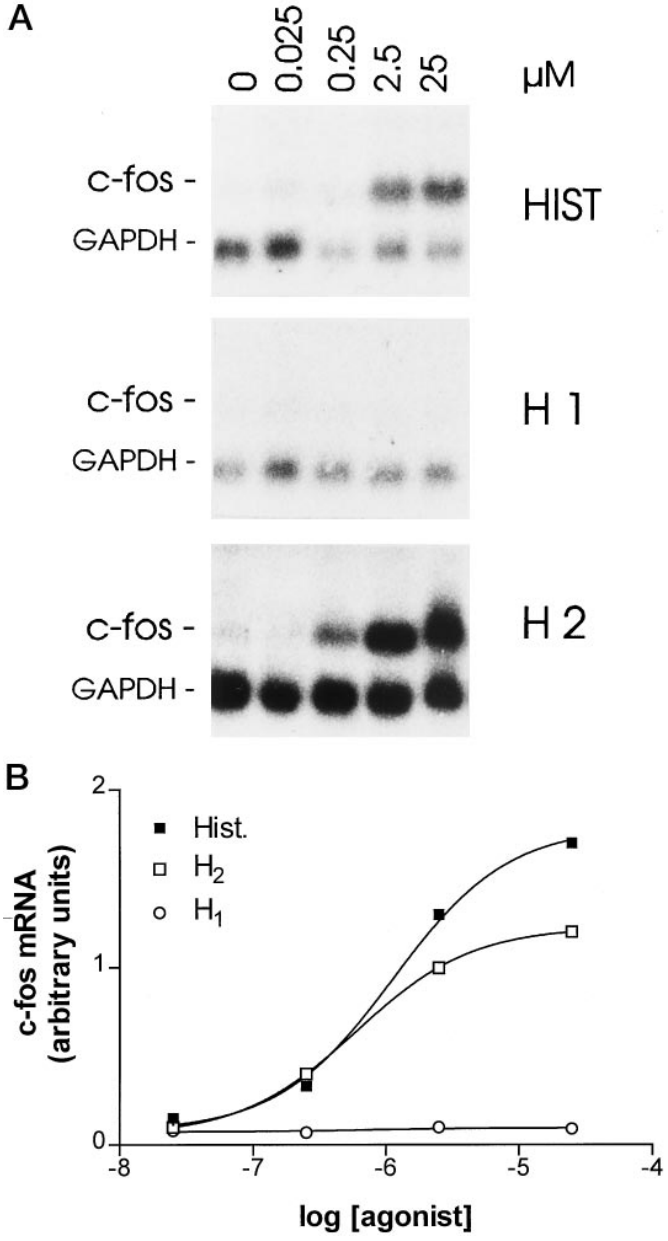

Fig. 2. Concentration-dependent effects of histamine, H_1 agonist, and H_2 agonist on c-*fos* mRNA levels. U937 cells were incubated 1 hr with increasing concentrations of histamine (*HIST*), 2-(3 trifluormethylphenyl)histamine (*H1*), or dimaprit (*H2*). Total cellular RNA (20 μg) was hybridized to ^{32}P-labeled c-*fos* and GAPDH probes. A, autoradiograms. B, quantification of autoradiograms. Arbitrary units represent the ratio of c-*fos* mRNA to GAPDH mRNA. Data are representative of at least three independent experiments.

GAPDH transcript were essentially constant throughout the assay.

Dose-response analysis of the effects of histamine and the H_1 and H_2 agonists on the quantitative levels of c-*fos* mRNA. Concentration-response curves were performed after 1-hr incubation in the presence of histamine or the H_2 agonist. Both agents induced dose-dependent increases in the levels of c-*fos* mRNA (Fig. 2, *top*). EC_{50} values and doses required to obtain maximal response were obtained by densitometric analysis of the autoradiograms. The EC_{50} values for histamine and dimaprit were 1.4 $\pm$ 0.3 and 0.6 $\pm$ 0.1 μM, respectively, whereas the maximal response was obtained with doses of >10 μM for both reagents (Fig. 2, *bottom*). Again, no change was observed in cells treated with the H_1 agonist.

The increase in c-*fos* expression is induced specifically via the H_2 receptor. A combined treatment with the agonist and antagonists showed that only H_2 receptors were directly linked to the increases in c-*fos* levels. As shown in Fig. 3, the effect of histamine was completely blocked by famotidine, an H_2 antagonist, whereas no changes were seen with pyrilamine, an H_1 antagonist. These observations were reinforced by the fact that famotidine, but not pyrilamine,

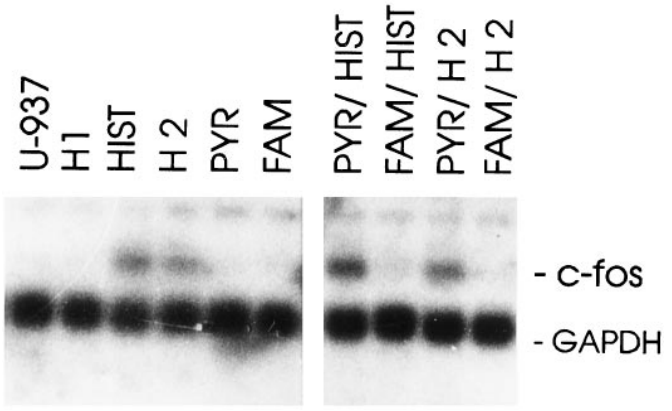

Fig. 3. Effects of H_1 and H_2 antagonists on histamine-induced c-*fos* expression. U937 cells were pretreated 5 min with 10 μM pyrilamine (*PYR*) or 10 μM famotidine (*FAM*). Cultures were continued 1 hr in the presence of histamine (*HIST*), 2-(3-trifluormethylphenyl)histamine (*H1*), or dimaprit (*H2*) (10 μM each). Total cellular RNA (20 μg) was hybridized with ^{32}P-labeled c-*fos* and GAPDH probes. Data are representative of at least three independent experiments.

abrogates the increase in c-*fos* induced by the H_2 agonist. Neither pyrilamine nor famotidine was able to alter c-*fos* expression alone.

The stimulatory effect of dimaprit on c-*fos* mRNA levels is mediated by PKA activation. To investigate whether protein phosphorylation is involved in the mechanism of c-*fos* modulation, a number of assays were carried out in which H7, a PKA/PKC inhibitor (27), was included before the addition of histamine or H_2 agonist to U937 in culture. The results shown in Fig. 4 demonstrate that H7 dramatically blocked the increase in c-*fos* mRNA levels evoked by histamine or dimaprit. In addition, dbcAMP, a PKA activator, induced an increase in c-*fos* transcript levels that could be blocked by H7. BM, a specific PKC inhibitor (28), did not modify c-*fos* expression induced by histamine or dimaprit but instead completely abolished the increase in c-*fos* mRNA evoked by PMA, a well-known PKC activator (29). These results suggest that protein phosphorylation mediated by PKA is part of the mechanism involved in histamine-induced c-*fos* gene expression.

c-*myc* is not affected by histamine. As indicated in Fig. 5, U937 cells express the c-*myc* gene. This expression was

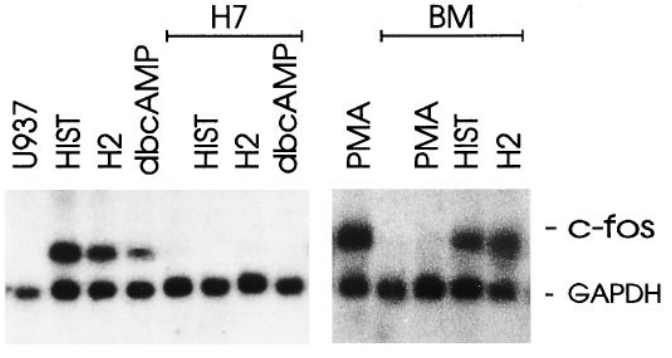

Fig. 4. Effects of H7 and bisindolylmaleimide on c-*fos* expression. U937 cells were pretreated 20 min with 20 μM H7 or 0.5 μM BM. Cultures were continued 1 hr in the presence of 10 μM histamine (*HIST*), 10 μM dimaprit ($_{H2}$), 0.4 mM dbcAMP, or 10 nM PMA. Total cellular RNA (20 μg) was hybridized with ^{32}P-labeled c-*fos* and GAPDH probes. Data are representative of at least three independent experiments.

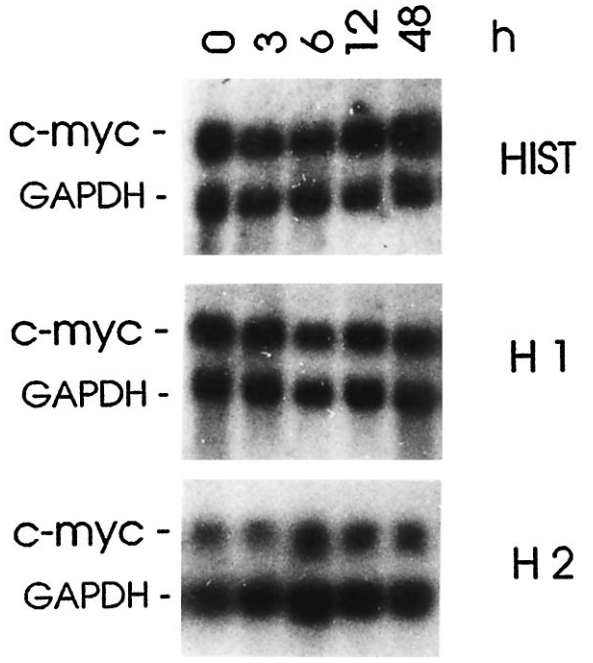

Fig. 5. Kinetics analysis of the effects of histamine, H_1 agonist, or H_2 agonist treatment on c-*myc* mRNA levels. U937 cells were cultured in the presence of histamine (*HIST*), 2-(3-trifluormethylphenyl)histamine (*H1*), or dimaprit ($_{H2}$) (10 μM each) for the indicated periods of time. Total cellular RNA (20 μg) was hybridized to ^{32}P-labeled c-*myc* and GAPDH probes. Data are representative of at least three independent experiments.

studied after the addition of 10 μM concentration of histamine, H_1 agonist, or H_2 agonist. During different periods of time (1–48 hr), no variations in the expression of this oncogene could be observed. Prolonged treatments of $\leq$6 days gave similar results (data not shown).

Histamine and H_2 agonist induce cAMP levels in U937 cells. Histamine and dimaprit produced a dose-dependent increase in cAMP levels, with a maximum accumulations of ~100-fold on the basal value for histamine and >50-fold for dimaprit, with EC_{50} values of 1.8 $\pm$ 0.2 and 0.7 $\pm$ 0.2 μM, respectively (Fig. 6). The EC_{50} values for histamine and the H_2 agonist that produced increase in cAMP levels were found to coincide with the EC_{50} values that induce c-*fos* expression (Figs. 2, *bottom*, and 6).

c-*fos* and c-*myc* levels are regulated by dbcAMP. U937 cells in culture were supplemented for various period of times with 0.4 mM dbcAMP, after which both c-*fos* and c-*myc* mRNA levels were examined. As indicated in Fig. 7, dbcAMP remarkably increased c-*fos* mRNA level within the first hour, and the level remain elevated for $\geq$12 hr after treatment. This sustained effect was not observed using histamine or the H_2 agonist. Under similar conditions, the levels of c-*myc* mRNA were markedly reduced within 1 hr of treatment with dbcAMP.

Levels of Fos and Myc proteins in U937 cells treated with H_2 histamine agonist and dbcAMP. Kinetic analysis of Fos and Myc protein content was carried out on cells treated with dimaprit or dbcAMP. Translation was found to follow a pattern that paralleled the corresponding oncogene mRNA levels. Dimaprit produced a transient increase in Fos protein with a maximal response after 2 hr, which remained higher than controls for $\geq$6 hr and progressively decreased to

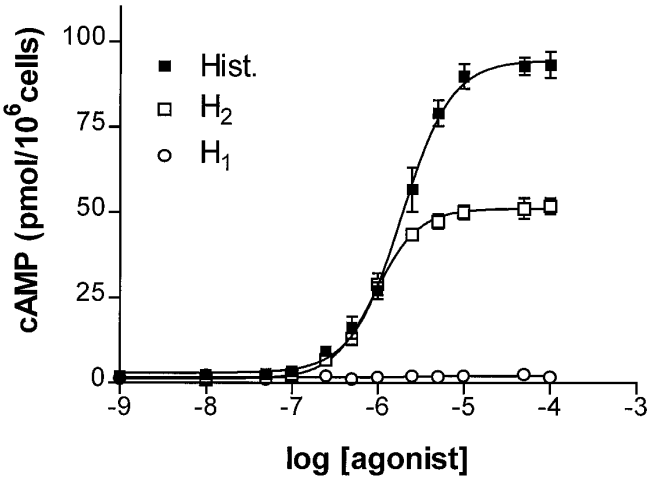

Fig. 6. Concentration-response curves for cAMP production. U937 cells were incubated with increasing concentrations of histamine (*Hist*), 2-(3-trifluormethylphenyl)histamine (H_1), or dimaprit (H_2) for 9 min at 37° in Hanks' medium supplemented with 1 mM IBMX, and cAMP levels were determined. Data are the mean ± standard error of triplicate assays. Similar results were obtained in at least four independent experiments.

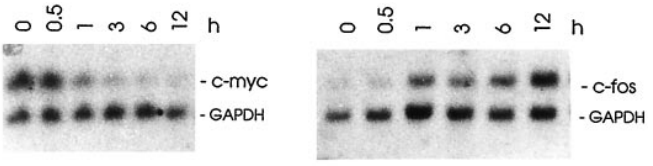

Fig. 7. Effect of dbcAMP treatment on c-*fos* and c-*myc* mRNA levels. U937 cells were cultured in the presence of 0.4 mM dbcAMP for the indicated periods of time. Total cellular RNA (20 μg) was hybridized to ^{32}P-labeled c-*myc* and GAPDH or to c-*fos* and GAPDH probes. Data are representative of at least three independent experiments.

basal concentrations after 12- 24 hr. In contrast, levels of this protein remain constantly elevated in cells exposed ≤24 hr to dbcAMP (Fig. 8). Myc protein levels were not affected by dimaprit in cells treated ≤24 hr but showed a remarkable and progressive decrease when cultured in the presence of dbcAMP (Fig. 8). Analysis of Jun protein levels showed a behavior similar to the one found for Fos in cells treated with dimaprit or dbcAMP (Fig. 8).

Histamine does not induce changes in cell proliferation. Fig. 9 illustrate the results on U937 cell proliferation after 3 days of treatment with histamine, H_1 or H_2 agonist, or dbcAMP at the indicated concentrations. Neither histamine nor its agonists were able to inhibit cell division. In contrast, dbcAMP inhibited U937 cell proliferation. This effect was dose dependent, exhibiting an EC_{50} value of 116 ± 12 μM. Forskolin showed a similar inhibitory effect with an EC_{50} value of 19 ± 6 μM.

H_2 agonist has no effect on cell differentiation. Essentially all cells remained functionally undifferentiated after 3 days of treatment with dimaprit (Table 1). As expected, cell differentiation takes place in the presence of dbcAMP. This was judged by the ability of dbcAMP to promote reduction of NBT, to increase migration rates of cells challenged with the chemoattractant rhC5a, and to induce the expression of CD14 and CD11b myeloid differentiation cell surface antigens (Table 1). Forskolin, an inducer of the PKA pathway, showed similar results to dbcAMP (data not shown).

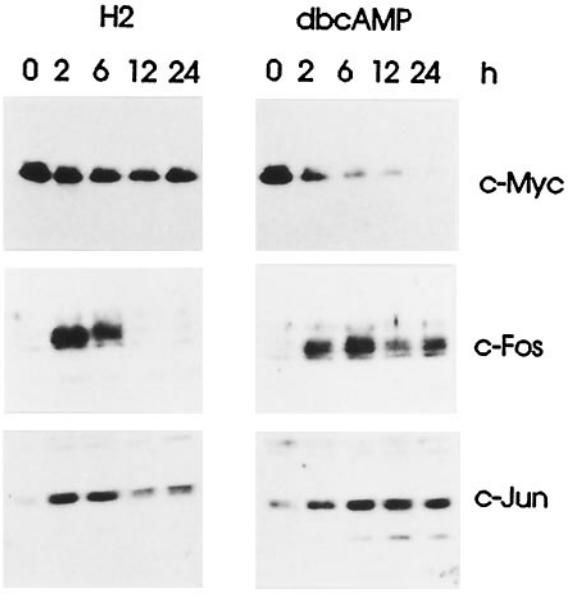

Fig. 8. Time-dependent effects of dimaprit and dibutyryl cAMP on Fos, Myc, and Jun protein levels. U937 cells were incubated with 10 μM dimaprit or 0.4 mM dbcAMP for the indicated periods of time before harvest and lysis as described in Materials and Methods. Samples were electrophoresed in 12% SDS-polyacrylamide gels, transferred to nitrocellulose, and immunoblotted with polyclonal purified rabbit serum against Fos, Myc, and Jun. Data are representative of at least three independent experiments.

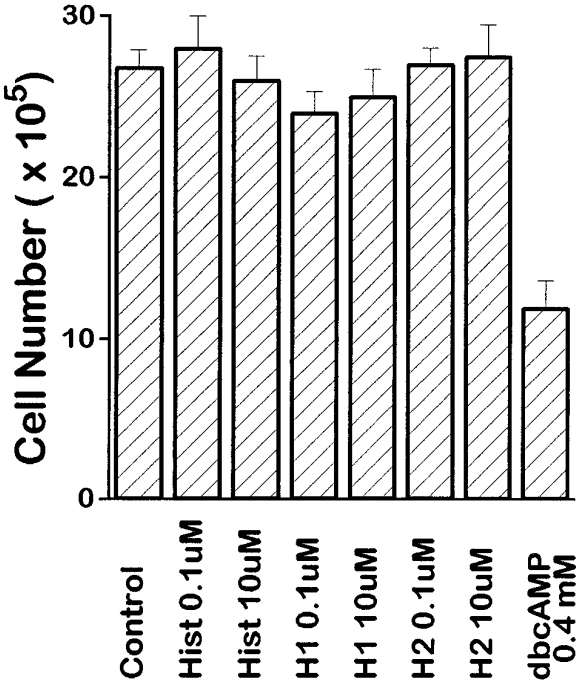

Fig. 9. Effects of histamine, H_1 and H_2 agonists, and dbcAMP in U937 cell proliferation. U937 cells were cultured with histamine (*Hist*), 2-(3-trifluormethylphenyl)histamine (*H1*), dimaprit (*H2*), or dbcAMP and treated for 3 days. Cells were harvested, and an aliquot was counted using an hemocytometer chamber. Data are the mean ± standard error of assay quadruplicates. Similar results were obtained in at least three independent experiments.

Histamine H_2 receptor desensitization. The susceptibility of the histamine H_2 receptor to desensitization was further investigated in this cell line. Results showed a dra-

TABLE 1

Effects of dimaprit and dbcAMP on U-937 cell differentiation

U937 cells were treated for 72 hr with 10 μM dimaprit or 0.4 mM dbcAMP. The proportion of chemoattracted cells, induction of cell surface markers for myeloid differentiation, and NBT-reducing activity were performed as described in Materials and Methods. Data are the mean ± standard error of assay quadruplicates. Similar results were obtained in at least three independent experiments.

	Chemotaxis assay migrated cells	Cell surface marker-positive cells		NBT-positive cells
		CD14	CD11b	
		%		
Control	0.4 ± 0.1	6.3 ± 3.3	34.9 ± 5.0	4.2 ± 2.9
Dimaprit	1.2 ± 0.1	8.5 ± 3.1	37.3 ± 3.4	3.9 ± 0.1
dbcAMP	41.0 ± 2.1[a]	25.1 ± 4.5[a]	68.9 ± 1.7[a]	35 ± 3.2[a]

[a] $p < 0.002$ relative to control.

matic reduction in cAMP production by the H_2 agonist when U937 cells were pretreated with dimaprit. In fact, the agonist could no longer induce a response when cells were pretreated for 2 hr with 10 μM concentration of the H_2 agonist. Half-maximal desensitization was observed at 20 ± 3 min after cell preexposure (Fig. 10). To establish whether desensitization was restricted to the histamine H_2 receptor, PGE_2-mediated cAMP induction was examined in cells pretreated with dimaprit. PGE_2 increased cAMP production in the U937 cell line for ≥2 hr. Identical assays carried out in the presence of forskolin showed similar results (Fig. 10, *inset*).

Discussion

The current study reports the effects of histamine on the levels of two nuclear proto-oncogenes, c-*fos* and c-*myc*, in U937 cells. Results indicate that histamine and dimaprit induce a transient increase in the levels of c-*fos* that is specifically mediated through H_2 receptors.

The presence of this type of receptor in U937 cells was reported in a study by Gespach *et al.* (10), in which H_2 receptors exhibit a classic pharmacological pattern described in the right atrium of guinea pig. In addition, the cAMP response to H_2 receptor stimulus obtained in the current study was concordant with data reported in the literature (1).

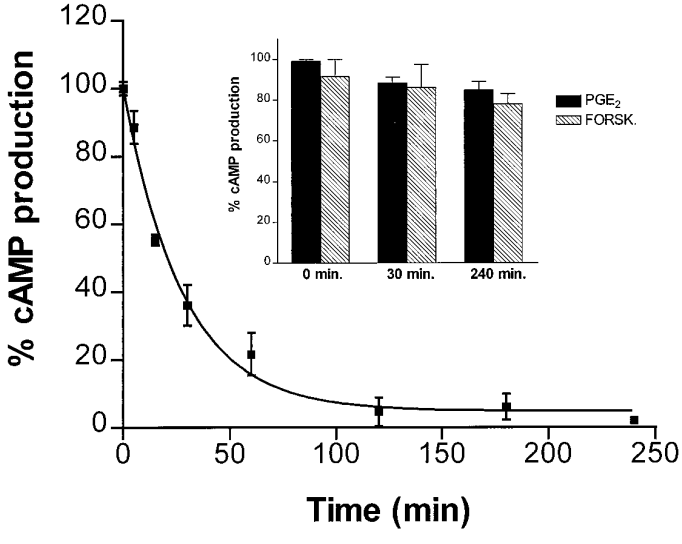

Fig. 10. Dimaprit induces desensitization of the histamine H_2 receptor. U937 cells were preincubated for different period of times with 10 μM dimaprit, further washed, and stimulated with dimaprit in the presence of 1 mM IBMX; cAMP production was determined as described in Materials and Methods. *Inset*, Cells pretreated with dimaprit for 0, 30, and 240 min were stimulated with 1 μM PGE_2 or 10 μM forskolin (*FORSK*), and cAMP production was determined. Data are shown as the percentage of cAMP production in response to dimaprit in function of the basal level in nonpretreated cells and are the mean ± standard error of assay triplicates. Similar results were obtained in at least three independent experiments.

The demonstration that the EC_{50} values for cAMP and c-*fos* expression were similar suggests that this increase is modulated via cAMP-PKA. Studies performed in the presence of H7 showed dramatic blocking of the effects of histamine and its agonist on the expression of this proto-oncogene.

Previous studies performed by our group demonstrated that histamine modulates the turnover of phosphoinositides in U937 cells in a dose-dependent manner through H_1 receptors (9). In addition, the regulation of PKC activity by the activation of this pathway via H_1 receptors has been described (1). Furthermore, the existence of diverse PKC isoenzymes with activity for distinct substrates *in vitro* has revealed differences that may indicate an even more pronounced specificity toward natural substrates under physiological conditions (29). Therefore, unlike PMA, possibly histamine or H_1 agonists activate PKC isoforms that are unable to stimulate c-*fos* transcription via PKC. The fact that the H_1 agonist did not induce changes in c-*fos* mRNA levels supports the idea that the effect of histamine at this level is independent of PKC. In addition, pretreatment with bisindolylmaleimide, a selective PKC inhibitor, did not alter the expression of the c-*fos* proto-oncogene increased by either histamine or dimaprit. This allows us to conclude that the intracellular increase in cAMP through H_2 receptors, together with the increase in PKA activity, might be responsible for the increased levels of c-*fos* mRNA. This is supported by the fact that dbcAMP, a direct activator of PKA, also increased c-*fos* expression and can be blocked by H7.

The induction of c-*fos* by cAMP has been demonstrated in numerous systems. The characteristics of this control are partly cell-type specific, as illustrated by the very high and stable transcription levels of c-*fos* in cAMP-stimulated macrophages and a weaker and transient effect in other cells (30). Enhanced c-*fos* expression associated with an increase in the intracellular concentration of cAMP have been described in systems in which this cyclic nucleotide can induce cellular proliferation [e.g., Swiss 3T3 cells (31) and dog thyroid cells (32)].

Contrarily to the response of c-*fos*, we did not detect modifications in the expression of the c-*myc* proto-oncogene in cells treated with histamine or its agonists. These results differ from those described for other hematopoietic cell lines in which intracellular increases in cAMP by histamine in-

duced a pronounced down-regulation of c-*myc* expression (3, 33). However, using a membrane-permeable analogue of cAMP, dbcAMP, we have shown an increase in c-*fos* transcript concomitant with a down-regulation of c-*myc* expression. Similar results concerning c-*myc* expression were previously reported in HL-60 cells (34). It is not clear whether butyrate, a putative hydrolytic product of dbcAMP, contributes to U937 cell differentiation. Exogenously added sodium butyrate does not affect c-*fos* mRNA expression (35) or induce morphological or functional alterations characteristic of differentiated phagocytes (36). Consequently, further studies are needed to clarify this issue. However, using forskolin to induce accumulation of intracellular cAMP, we observed in U937 cells the appearance of several characteristics of differentiated cells, disregarding any participation of butyrate (data not shown).

Because gene expression regulation in general is not restricted to mRNA levels alone (37), the effects of H_2 agonist and dbcAMP on Fos, Jun, and Myc protein expression were also studied. Dimaprit transiently elevated Fos levels, whereas dbcAMP produced increases for longer periods of times. In addition, we observed that the levels of Jun protein, the other component of the activator protein-1 complex, in cells treated with H_2 agonist or dbcAMP showed similar patterns. A great deal of circumstantial evidence suggests that control of Myc function may be important during differentiation. Heightened Myc expression in murine erythroleukemia cells and monocytes can block differentiation (38). It appears that the transient elevation in cAMP induced by H_2 agonist is insufficient to down-modulate Myc protein expression over the long term, whereas in cells incubated in the presence of dbcAMP, we clearly observed a significant and sustained reduction in Myc protein levels.

In many G protein-coupled systems, such as histamine receptors, prolonged exposure to agonists leads to a state of reduced responsiveness to these agents, a process termed "desensitization" (39). In this regard, we show that the desensitization of H_2 receptors in U937 cells exhibits a half-life of 20 min and that by 120 min this process is complete. Furthermore, desensitization proved to be homologous because cells pretreated with dimaprit showed an intact cAMP response to both PGE_2 and forskolin. This is in agreement with the transient increase in c-*fos* expression and supports the hypothesis that this effect is mediated by cAMP. On the other hand, this rapid desensitization followed by a decrease in cAMP levels could be the basis for the lack of response in c-*myc* expression. In other cellular systems in which the desensitization of the H_2 receptor is retarded, histamine and its agonists down-regulate the levels of c-*myc* gene expression (40).

We demonstrate here that histamine is incapable of inhibiting U937 cell proliferation despite the noticeable though transient increase in cAMP levels, whereas an important inhibition by dbcAMP could be observed. Accordingly, we have shown that forskolin and dbcAMP, but not dimaprit, induced a significant proportion of cells to acquire the ability to (i) produce superoxide anion, (ii) express cell surface maturation markers, and (iii) increase chemotaxis. Taken together, these results indicate that the binding of agonist to H_2 histamine receptors triggers an early specific response characterized by an increase in cAMP levels, but their rapid desensitization makes this response insufficient to induce differentiation of U937 cells. It could be possible that in promonocytic U937 cells, but not in normal progenitors, desensitization via H_2 receptors plays a significant role in reducing their capacity to reach terminal cell differentiation. Supporting this idea, although not rigorously related to this cell hierarchy, histamine has been reported to cooperate with granulocyte-colony stimulating factor in the induction of normal cell differentiation (40). Therefore, a better understanding of this issue could make possible new avenues in therapeutic intervention.

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