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H_1 and H_2 histamine receptors mediate the production of inositol phosphates but not cAMP in human breast epithelial cells

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Abstract. *Objective:* In the present work we studied the H_1 and H_2 histamine receptor expression and function in HBL-100 and MCF-10A cells, derived from non-tumorigenic human breast epithelia, and in MCF-10T, the H-ras-transfected MCF-10A counterpart. The signal transduction pathways associated with these receptors, and the expression of proto-oncogenes c-fos, c-myc and c-jun at the mRNA and protein levels, were examined.
Results: Saturation analysis using intact cells, showed two binding sites for [^{3}H]tiotidine and [^{3}H]mepyramine. Pretreatment of purified membrane with guanosine 5′-γ thiotriphosphate resulted in the loss of the low affinity component for [^{3}H]tiotidine binding, and of the high affinity component for [^{3}H]mepyramine. In both cases, there was no modification in the total number of sites for both ligands. Neither H_1 nor H_2 agonists stimulated cyclic AMP production, though this pathway is functional in these cells. On the other hand, both H_1 and H_2 agonists enhanced phosphoinositide turnover in a dose-dependent fashion, and this induction is pertussis toxin-insensitive. H_1 and H_2 agonists did not influence the expression of c-myc or c-fos mRNA, nor their encoded proteins.
Conclusions: These results indicate that the three cell lines examined showed functional H_1 and H_2 histamine receptors, which are involved in the metabolic turnover of inositol phosphates but are ineffective in the modulation of the cyclic AMP response. The fact that H_2 receptors have lost their ability to stimulate cyclic AMP production would imply the loss of a regulatory mechanism of cell growth.

Key words: Signal transduction – C-myc – C-fos – Human breast epithelial cells

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Introduction

Based on the differences in the pharmacological profile of several drugs, three histamine receptors (H_1, H_2 and H_3) can be distinguished [1]. Molecular biology studies indicate that the histamine H_1 and H_2 receptors belong to the large multigene family of G protein-coupled receptors [2, 3]. Structurally, these receptors are characterized by seven transmembrane α-helices, and functionally, by their ability to transmit signals to effector molecules via GTP-binding regulatory proteins [4]. It is generally accepted that the H_2 receptor is coupled to the adenylyl cyclase system through direct interaction with a Gs protein family [1]. Recently, a number of new signaling pathways have been described for the H_2 receptor, including H_2 receptor-dependent Ca^{2+} mobilization [5], probably due to the activation of phospholipase C [6]. At present there is evidence that this particular process would be mediated by the Gq protein family [7].

On the other hand, it is well characterized that histamine H_1 receptors produce functional responses, including phosphoinositide turnover, due to the activation of phospholipase C, via the Gq protein family [7].

We have reported that N-nitroso-N-methylurea induced experimental mammary carcinomas exhibit H_1 and H_2 histamine receptors with an atypical linkage to signal transducers [8]. The expression of an H_2 receptor coupled to enhancement of phosphoinositide turnover in cells with an activated ras oncogene, enables histamine to stimulate cell growth [9]. Moreover, histamine regulates histidine decarboxylase expression and endogenous synthesized histamine is released to the extracellular medium, acting as an autocrine growth factor [10, 11]. In these experimental tumors, treatment with H_2 antagonists produces a significant inhibition of growth when tested both in vivo and in vitro in primary cultures of rat mammary tumor cells [11, 12]. Furthermore, carcinomas

and benign lesions of the human mammary gland express histamine receptors [13], and changes in the normal association of H_2 receptors to transductional pathways are observed as a consequence of the neoplastic transformation [14].

The aim of the present study was to evaluate H_1 and H_2 histamine receptors expression and function in HBL-100 and MCF-10A cells, derived from non-tumorigenic human breast epithelia, and in MCF-10T, the H-ras-transfected MCF-10A counterpart. The signal transduction pathways associated with these receptors, and the expression of proto-oncogenes c-fos, c-myc and c-jun at the mRNA and protein levels, were examined.

Materials and methods

Materials

[^{3}H]tiotidine, [^{3}H]*myo*-inositol and [^{3}H]cAMP, were purchased from New England Nuclear (Boston, MA, USA). Histamine dihydrochloride, mepyramine hydrochloride, ranitidine, famotidine, inositol, cAMP, cholera toxin, pertussis toxin, forskolin, GTPγS (guanosine 5′-γ thiotriphosphate), IBMX (3-isobutyl-1-methylxanthine), ACS grade salts, cell culture grade reagents and media, were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Dowex AG-1X8 formate form was from Bio-Rad Laboratories (Richmond, CA, USA). Electrophoresis grade reagents and culture sera were purchased from Gibco-BRL (Bethesda, MD, USA). The H_1 agonist 2-(3-(Trifluormethyl)phenyl)-histamine and the H_2 agonist dimaprit, were generously provided by Dr. W. Schunack from Freie Universität, Berlin, and Dr. A. Buschauer, from Regensburg Universität, Germany.

Cell culture

The MCF-10A and HBL-100 non-tumorigenic human breast epithelial cells lines, and the tumorigenic MCF-10T cell line, derived from MCF-10A transfected with the activated H-ras gene [15], were employed.

MCF-10A cells, provided by Dr. S. Gutkind (LCDO, NIDR-NIH, Bethesda, USA) were routinely grown in DME/F12 medium supplemented with 5 % horse serum, 10 ng/ml EGF, 5 µg/ml insulin, 0.5 µg/ml hydrocortisone, 100 ng/ml cholera toxin and 50 µg/ml gentamicine, as described by Soule et al [16]. MCF-10T cells, provided by Dr. J. Russo (Fox Chase Cancer Center, PA, USA), were maintained in the same medium supplemented with 100 µg/ml G-418. HBL-100 cells [17], purchased from the American Type Culture Collection (ATCC), were grown in RPMI 1640 containing 10 % fetal calf serum and gentamicine. All cell lines were cultured at 37 °C in a humidified atmosphere of 95 % air/5 % CO_2.

Receptor binding experiments

Confluent cell monolayers, grown in 24-well plastic dishes, were employed for the binding experiments. The H_2 antagonist [^{3}H]tiotidine, and the H_1 antagonist [^{3}H]mepyramine, were used as specific radioligand in concentrations ranging from 1 to 70 nM for [^{3}H]tiotidine and 1 to 200 nM for [^{3}H]mepyramine, and non-specific binding was determined using 100 µM of the corresponding unlabeled antagonist. The maximal inhibition of [^{3}H]tiotidine binding by: famotidine, histamine, ranitidine and amthamine, did not differ significantly from that obtained in the presence of 1 to 100 µM tiotidine, i. e., there was no displacement of the non-specific component which routinely account for 50–55 % of the total binding. The maximal inhibition of [^{3}H]mepyramine binding by: astemizole, loratadine, 2-(3-(Trifluoromethyl)phenyl)-histamine and ketotifen, did not differ significantly from that obtained in the presence of 1 to 100 µM mepyramine, i. e., there was no displacement of the non-specific component which routinely account for 5–10 % of the total binding (data not shown).

Incubations were performed in quadruplicate in 300 µl of 50 mM Tris-HCl buffer, pH 7.4, during 40 min at 4 °C, and terminated by gently washing with ice-cold buffer. Experiments performed on intact cells were carried out at 4 °C in order to avoid internalization of the ligand. Kinetic studies showed that equilibrium was reached after 30 min, and persisted for 4 h (data not shown). Bound radioactivity was extracted with 500 µl of ethanol, transferred to vials containing scintillation cocktail (HiSafe 3, Wallac), and radioactivity determined by liquid scintillation counting. Binding data was evaluated by use of LIGAND, a non-linear, weighted, least squares, curve-fitting procedure [18], and Scatchard plot analysis.

A purified membrane fraction was obtained by disrupting cells by sonication in 50 µM Tris-HCl buffer, pH 7.4, followed by 15 min centrifugation at 8500 × g. The supernatant was further centrifuged 40 min at 30000 × g, and the resulting pellet resuspended in a volume equivalent to the one used for intact cells. This preparation was exposed 30 min to 10 µM GTPγS at 37 °C, before binding.

Cyclic AMP measurements

Confluent cell monolayers were exposed 3 min at 37 °C in Hank's balanced solution with 1 mM IBMX, before incubating 9 min with different agonists. Incubations were stopped and extracted with ethanol, and 5 min centrifuged at 1200 × g. Supernatants were dried and resuspended in 50 mM Tris-HCl buffer, pH 7.4, to determine cAMP content by the competitive protein binding assay. Results are expressed as pmol of cAMP per 10^5 cells. Forskolin 10 µM and NaF 10 mM were employed as positive controls for cAMP production. Pretreatment with 1 µg/ml of cholera toxin was carried out at 37 °C for 2 h. Under these conditions, 50 % of the maximal response in cAMP production is obtained (data not shown).

For the determination of the concentration-response ratio, the following range of concentrations were employed: H_1 and H_2 histamine agonists, 10 nM to 10 µM; PGE_2, 1 nM to 10 µM; forskolin, 0.1 to 50 µM and NaF, 0.25 to 1 mM. Histamine antagonists were added at 10 µM concentration.

Phosphatidylinositol turnover

Cell monolayers at 60 % confluence were incubated 24 h at 37 °C with [^{3}H]*myo*-inositol (10 µCi/ml). Culture medium was changed after careful washing and cells were incubated 10 min with 10 mM LiCl. After 20 min of stimulation with different agonists, the incubation medium was removed, cell components were extracted with ethanol : chloroform : water 2 : 1 : 1, and the mixture was centrifuged 15 min at 1200 × g. Total [^{3}H]inositol phosphates present in the aqueous phase were obtained after anion-exchange chromatography. Eluted fractions were transferred to vials containing scintillation solution and radioactivity was determined by liquid scintillation counting. Results are expressed as the relation obtained when total [^{3}H]inositol phosphate activity was normalized to total [^{3}H]inositol recovered from the initial water wash of the columns corresponding to intracellular [^{3}H]inositol pool [19].

Total DNA content

To ascertain the cell number in the binding and cAMP experiments, cells grown in duplicate wells were lysed with 25 µl of 6 M guanidinium HCl, and total cellular DNA content was determined by fluorescence with Hoechst 33258 in a minifluorometer TKO 100 (Hoeffer Sci. Inst.), as instructed by the manufacturer. Cell number was determined from calibration curves for each cell line.

RNA isolation and northern blot analysis

Cells were grown to confluence in 100 mm dishes and starved 16 h previous to stimulation in DME/F12 supplemented with 1 mg/ml of BSA. Total RNA was isolated [20], and 15 µg aliquots were electrophoresed in 1% agarose-0.8 M formaldehyde gels, transferred to ZetaProbe membranes (Bio Rad), and hybridized overnight at 45 °C in 125 mM NaCl, 250 mM sodium phosphate pH 7.2, 7% SDS, 10% PEG, 50% formamide, and >10^6 cpm/ml of random-primed ^{32}P labeled probe. Filters were washed and exposed to AGFA Curix RP1 films. Hybridization to gapdh was used to ensure equal loading of RNA. The following probes were used: 1.8 Kb XhoI-EcoRI fragment of human c-fos cDNA cloned in pBK28 plasmid, donated by Dr. I. Verma (Salk Institute, USA), and a 1.2 Kb PstI fragment of gapdh, provided by Dr. A. Kornbliht (FCEN-UBA, Argentina). Plasmid containing fosB was donated by Dr. Rodrigo Bravo (Bristol-Myers, Squibb Pharmaceutical Research Institute, Princeton, N.J., USA) while the c-myc probe was a 1.4 Kb ClaI-EcoRI fragment from genomic DNA cloned in plasmid pHSR1 (ATCC).

Western blots

Cells grown in 60 mm dishes were starved 16 h in DME/F12 supplemented with 1 mg/ml of BSA before treatment and then lysed in 300 µl of 50 mM Tris-HCl, pH 6.8, 2% SDS, 100 mM 2-mercaptoethanol, 10% glycerol, and 0.05% bromophenol blue, and sonicated to shear DNA. Samples were then boiled 5 min, and 20 µl samples were electrophoresed in 12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. 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 of the specific primary antibody in 3% nonfat dried milk in TBST [21]. Rabbit sera against c-Myc, c-Jun, and c-Fos proteins were purchased from Santa Cruz Biochemicals (Santa Cruz, CA, USA). All subsequent washes were performed in TBST. Reactivity was developed using an anti-rabbit polyclonal antibody linked to horseradish peroxidase and enhanced chemiluminiscence reagents, according to the manufacturer's instructions (Amersham Pharmacia Biotech, UK).

Statistics

Results are expressed as the mean ± SEM of at least three independent experiments and statistical significance was analyzed by one-way ANOVA.

Results

Binding experiments

Saturation analysis using intact cells showed two sites for both [^{3}H]tiotidine and [^{3}H]mepyramine binding in the three cell lines. One site showed high affinity and low capacity, and the other lower affinity and high capacity (Table 1). When binding experiments for [^{3}H]tiotidine were performed with purified membrane fraction of HBL-100 cells previously incubated with 10 µM GTPγS, there was no modification in the total number of sites, but the high affinity component could no longer be observed (Q_{total} = 238 900 ± 24 980, mean ± S.E.M., n = 3) (Fig. 1A). In contrast, pretreatment with GTPγS for the [^{3}H]mepyramine assay resulted in the loss of the lower affinity component but, as for the [^{3}H]tiotidine binding, no modification in the total number of sites was detected (Q_{total} = 354 500 ± 34 900, mean ± SEM, n = 3) (Fig. 1B).

Table 1. Binding parameters in cell lines. Confluent cell monolayers grown in 24-well plastic dishes were employed for the binding experiments. The H_2 antagonist [^{3}H]tiotidine, and the H_1 antagonist [^{3}H]mepyramine were used as specific radioligands in concentrations ranging from 1 to 70 nM for [^{3}H]tiotidine and 1 to 200 nM for [^{3}H]mepyramine. The binding data were evaluated by use of LIGAND, a non-linear, weighted, least squares curve-fitting procedure. Data were calculated as the mean ± SEM of at least three independent experiments.

Cell line	[^{3}H]tiotidine		[^{3}H]mepyramine	
	B_{max} (sites/cell)	Kd (nM)	B_{max} (sites/cell)	Kd (nM)
MCF-10A	52100 ± 3200	3.3 ± 0.7	58000 ± 3500	14.3 ± 3.2
	260000 ± 31000	20 ± 3	303200 ± 41500	94 ± 5
MCF-10T	28000 ± 1200	1.8 ± 0.5	28700 ± 1800	11,5 ± 4.3
	110300 ± 21500	18 ± 2	290000 ± 23500	92 ± 8
HBL-100	45500 ± 5200	2.4 ± 0.6	55624 ± 4200	13,5 ± 2.3
	195000 ± 25000	21 ± 4	322100 ± 21200	97 ± 6

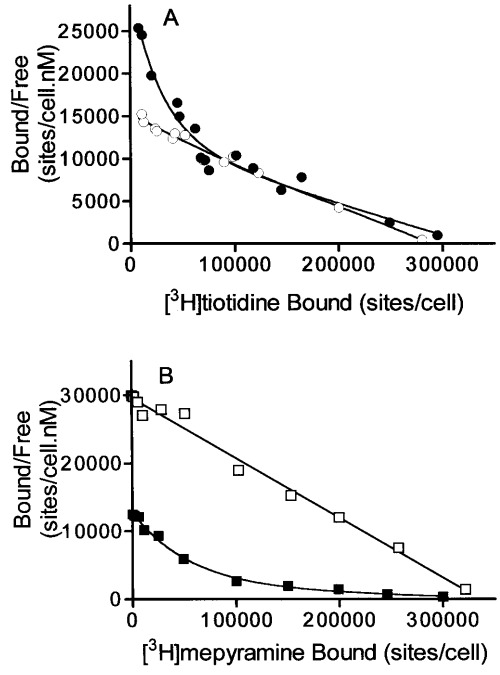

Fig. 1. A Comparative Scatchard plots from binding analysis in HBL-100 cells using [^{3}H]tiotidine. Open circles (○) correspond to a saturation experiment employing a purified membrane fraction from cells previously incubated with GTPγS 10 µM. Black circles (●) represent the binding data from the control membrane fraction of cells, where two sites with different affinity are present. Data are representative of three independent experiments. **B** Comparative Scatchard plots from binding analysis in HBL-100 cells using [^{3}H]mepyramine. Open squares (□) correspond to a saturation experiment employing a purified membrane fraction from cells previously incubated with GTPγS 10 µM. Black squares (■) represent the binding data from the control membrane fraction of cells, where two sites with different affinity are present. Data are representative of three independent experiments.

Cyclic AMP production

To study the transductional pathway associated with these receptors, we first analyzed cAMP production in response to histamine and its agonists. Data indicate that H_1 or H_2 agonists failed to produce any modification in cAMP concentra-

Table 2. cAMP production in cell lines. Concentration-response analysis for cAMP production in cells lines, 10 min stimulated with the H_2 agonist dimaprit, the H_1 agonist 2-(3-(Trifluormethyl)phenyl)-histamine, PGE_2 or forskolin used as control. Data were calculated as the mean ± SEM of at least three independent experiments. NR: no response.

Agent	MCF-10A		MCF-10T		HBL-100	
	EC_{50} (nM)	Stimulation (%)	EC_{50} (nM)	Stimulation (%)	EC_{50} (nM)	Stimulation (%)
H_1 Agonist	NR	NR	NR	NR	NR	NR
H_2 Agonist	NR	NR	NR	NR	NR	NR
PGE_2	45 ± 3	800	61 ± 13	500	34 ± 4	750
Forskolin	4975 ± 950	280	5560 ± 600	300	4730 ± 630	420

tion in the three cell lines investigated (Table 2). The same conclusion was reached for histamine alone or in the presence of H_1 or H_2 antagonists, independently of the dose. On the other hand, PGE_2 and forskolin gave rise to a dose-dependent increase in cAMP levels (Table 2). Similar results were obtained in the presence of NaF (data not shown).

In all three cell lines, pretreatment with cholera toxin produced an eight-fold increase in cAMP basal values (1.5 vs 12 pmol/10^5 cells) and potentiated the response to different concentrations of PGE_2 with no significant modification of the EC_{50} value (45 ± 9 nM) (Table 2, Fig. 2). However, neither H_1 nor H_2 agonists were able to modify cholera toxin basal values in MCF-10A cells (Fig. 2) nor in the other cell lines studied (data not shown). These results demonstrate the functionality of the metabolic cAMP pathway and clearly show that neither H_1 nor H_2 histamine receptors are coupled to adenylyl cyclase activation in MCF-10A, MCF-10T or HBL-100 cells.

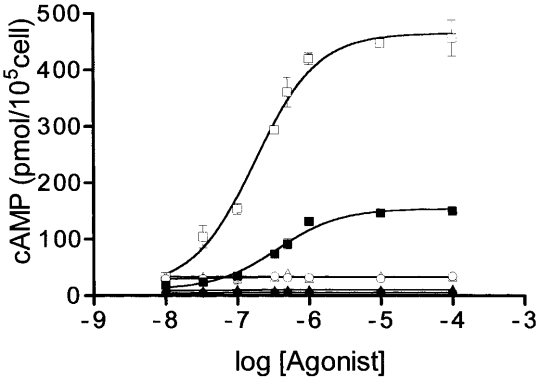

Fig. 2. Concentration-response analysis for cAMP production in MCF-10A cells, stimulated 10 min with the H_2 agonist dimaprit (●), the H_1 agonist 2-(3-(Trifluormethyl)phenyl)-histamine (▲), or PGE_2 (■) used as control. Open symbols represent the data corresponding to the concentration-response curves obtained after 2 h of preincubation with 1 µg/ml cholera toxin. Results were calculated as the mean ± SEM of at least three independent experiments.

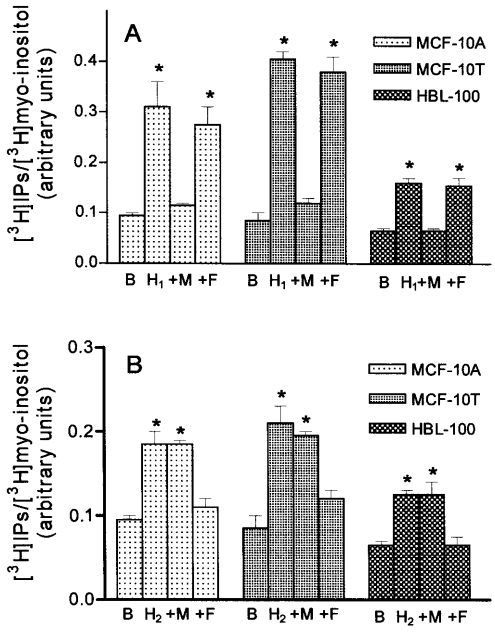

Fig. 3. **A** Total inositol phosphate production in cell lines exposed to 10 µM 2-(3-(Trifluormethyl)phenyl)-histamine (H_1), alone or in combination with 10 µM mepyramine (+M) or 10 µM famotidine (+F). Data shown are the means ± SEM of three independent experiments. Asterisks indicate significant differences compared to basal (B) conditions ($p < 0.001$, ANOVA). **B** Total inositol phosphate production in cell lines exposed to 10 µM dimaprit (H_2), alone or combined with 10 µM famotidine (+F) or 10 µM mepyramine (+M). Data shown are the means ± SEM of three independent experiments. Asterisks indicate significant differences compared to basal (B) conditions ($p < 0.001$, ANOVA).

Phosphatidylinositol turnover

In the three cell lines, histamine produced a concentration-dependent increase in phosphoinositide turnover with a similar EC_{50} (data not shown). When cells were stimulated with the specific agonists, both H_1 and H_2 receptors mediated this response (Table 3).

The H_1 agonist 2-(3-(Trifluormethyl)phenyl)-histamine produced a 3-fold increase in the levels of this second messenger (Table 3). This response was completely abolished by mepyramine (H_1 antagonist), while 10 µM famotidine (H_2 antagonist) did not inhibit the stimulation of phosphatidyli-

Table 3. Phosphatidylinositol turnover in cell lines. Concentration-response analysis for inositol phosphate production in cell lines stimulated with the H_2 agonist dimaprit or the H_1 agonist 2-(3-(Trifluormethyl)phenyl)-histamine. Data were calculated as the mean ± SEM of at least three independent experiments.

Agent	MCF-10A		MCF-10T		HBL-100	
	EC_{50} (nM)	Stimulation (%)	EC_{50} (nM)	Stimulation (%)	EC_{50} (nM)	Stimulation (%)
H_1 agonist	250±75	100	194±14	150	270±45	97
H_2 agonist	200±25	200	247±45	300	193±30	195

nositol turnover produced by H_1 agonists (Fig. 3A). In a similar way, the H_2 agonist dimaprit produced a dose-dependent increase in phosphatidylinositol hydrolysis (Table 3). Famotidine (10 μM) selectively inhibited H_2 agonists-mediated elevation of intracellular inositol phosphates, while mepyramine failed to prevent this response (Fig. 3B). Pretreatment with different concentrations of pertussis toxin did not induce modifications in the phosphatidylinositol turnover generated by both H_1 and H_2 agonists, similar to results described for MCF-10A (Fig. 4), MCF-10T, and HBL-100 cells (data not shown), indicating that the G protein involved in this signal transduction pathway is pertussis toxin-insensitive, and presumably does not belong to the Gi family.

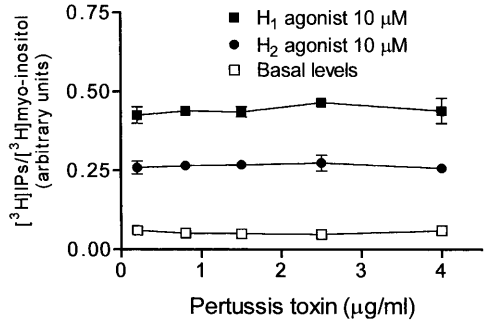

Fig. 4. Effects of pertussis toxin on phosphatidylinositol turnover in the MCF-10A cell line. Cell membranes were pretreated 60 min with pertussis toxin at various concentrations, and the effect of agonists was evaluated under conditions of maximal response. Data shown are the means ± SEM of four independent experiments.

Expression of proto-oncogenes coding for transcription factors

Analysis of c-fos and fosB mRNA expression was performed in the three cell lines after addition of H_1, H_2 histamine agonists, or the combination of both. No effect was observed under these conditions. Fig. 5A shows a typical result from the three cell lines after 45 min of incubation with 1 μM of each agonist. In contrast to this observation, EGF, a mitogen known to induce the expression of several proto-oncogenes which codify for transcription factors, produced a remarkable increase in the levels of c-fos and fosB expression (Fig. 5A), and in c-myc mRNA (data not shown). In addition, kinetics studies performed on c-Fos, c-Jun and c-Myc protein expression gave results similar to those reported for their respective transcripts in MCF-10A (Fig. 5B), MCF-10T, and HBL-100 cells (data not shown).

Discussion

The present results confirm the existence of functional histamine receptors in MCF-10A and HBL-100 cell lines, derived from non-tumorigenic human breast tissue and MCF-10T (MCF-10A with an activated H-ras oncogene) with neoplasic characteristics.

Ligand binding analysis revealed the presence of a high affinity population of H_2 receptors in these cells, in addition to the unique site of relatively low affinity, usually reported in other systems. [^{3}H]tiotidine single binding sites with a Kd of 10 nM have been identified in different tissues [8, 13, 22–25] and cell lines [26, 27]. The presence of a double site for [^{3}H]tiotidine, and the modification of the binding profile after treatment with GTPγS, suggests the existence, even in the absence of ligand, of a preformed G protein-receptor complex similar to the one described for other members of the G protein-coupled receptors family [28]. In most systems, the percentage of this activated receptor is very low and difficult to identify; but in these cells, a large fraction of this component could be observed, possibly due to H_2 receptor overexpression [29]. In addition, the H_2 receptor could be coupled to a different G protein, which may be expressed in a higher extent in these cells, thus contributing to the high proportion of this activated state.

The fact that an antagonist shows a high affinity binding site for the receptor-G protein complex, cannot be explain-

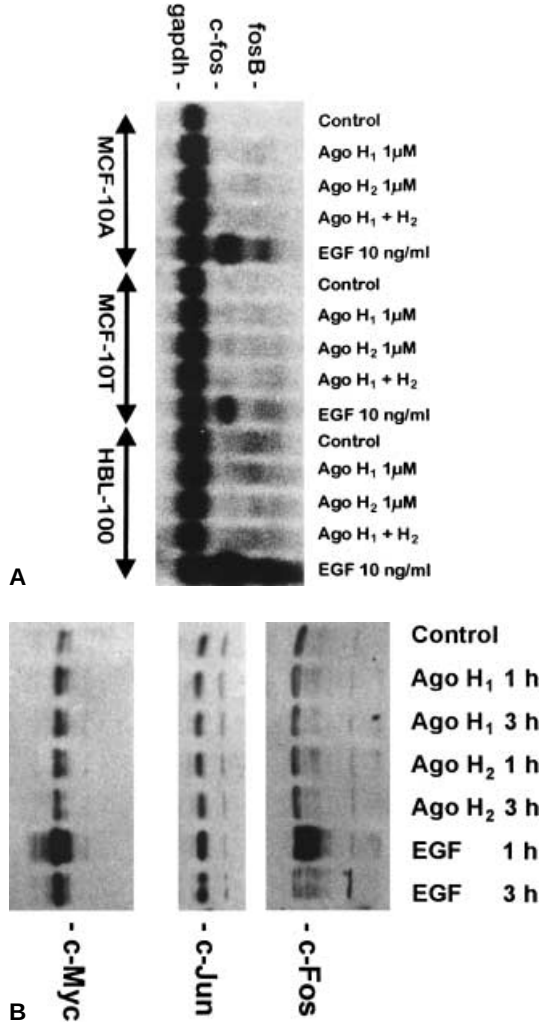

Fig. 5. **A** Analysis of c-fos and fosB proto-oncogene mRNA levels in MCF-10A, MCF-10T, and HBL-100, after 45 min of treatment with EGF, H_1, or H_2 agonists. Hybridization to gapdh (glyceraldehyde-3-P dehydrogenase) probe was used as control. Data are representative of three independent experiments. **B** Effects of EGF, H_1, or H_2 agonists on c-Fos, c-Jun, and c-Myc proteins levels in MCF-10A cells. Data are representative of three independent experiments.

ed by most classical interaction models, such as the allosteric ternary complex model [30], or the two state model [31]. Computer simulations using the cubic ternary complex model [32] show that a ligand which exhibits greater affinity for the receptor-G protein complex than for the uncoupled receptor, could be an agonist, an antagonist or an inverse agonist (to be published elsewhere).

Binding assays with [^{3}H]mepyramine also disclosed high- and lower-affinity states for H_1 receptors, and showed a profile expected for an inverse agonist, in which the lower affinity component disappears after pretreatment with GTPγS [33]. Once again, this shift to a higher affinity state could be explained by the same model.

Stimulation of H_1 receptors with specific agonists resulted in an enhancement of phosphoinositide turnover, and this effect was selectively blocked by H_1 antagonists. This response proved not to be sensitive to pertussis toxin indicating that an insensitive G protein is involved in this mechanism, and probably belongs to the Gq family. Furthermore, experimental determination of cAMP intracellular levels showed that H_1 receptors are not coupled to adenylyl cyclase. Collectively, these results indicate that the three cell lines examined present typical H_1 histamine receptors [1].

The present study demonstrates that histamine or its agonists failed to modulate intracellular levels of cAMP in the three cell lines examined. Nevertheless, results obtained using forskolin, NaF, PGE_2 and cholera toxin, suggest that this transductional pathway is functional in these systems.

Analysis of the second messenger pathways activated by the H_2 agonists resulted in an elevation of intracellular inositol phosphates in all three cell lines. The selective blockage by an H_2 antagonist and the EC_{50} values for the different agonists indicate that, in this cells, H_2 receptors are linked to phosphoinositide turnover rather than cAMP induction. The expression of diverse G proteins may be involved in this atypical receptor linkage. Although the adenylyl cyclase pathway is functional, the lack of stimulation of cAMP production by H_2 receptors might be explained as a consequence of the intracellular balance of the different G proteins [34]. Our results could also be in agreement with the concept of receptor promiscuity where in some situations, such as receptor overexpression, anomalous receptor coupling has been described [35]. Recently, an allelic variation of the human histamine H_2 receptor has been identified, showing six single base changes from the original sequence, suggesting critical effects upon second messenger activation by this receptor [36]. It would be interesting to evaluate which subtype of H_2 receptor gene is expressed in these cells, and the G protein linked to this receptor.

On the other hand, the induction of proto-oncogene products such as the Fos, Jun, and Myc families are of great importance in the cell cycle progress. Different factors modulate their expression, including ligand-receptor interaction, second messenger production, and activation of protein kinases, which in turn activate specific transcription factors [37]. As indicated above, we were unable to demonstrate any significant effect of histamine agonists on mRNA and protein levels of proto-oncogenes involved in cell growth and differentiation, including c-fos, fosB, c-myc, and c-jun.

In these cell lines, insulin and EGF promote phosphoinositide turnover, although only EGF, which acts mainly through activation of the MAP kinase pathway, was capable of inducing the expression of these proto-oncogenes (unpublished results). Therefore, our results suggest that modulation in the phosphoinositide pathway is not enough to promote induction of proto-oncogene expression in these systems. In addition, the transfection of MCF-10A with an activated H-ras oncogene, which confers neoplastic characteristics, such as anchorage-independent proliferation and low growth factors requirement, do not interfere with the histamine receptors-driven signal transduction.

In conclusion, the present study indicates that breast cell lines derived from non-tumorigenic origin, with high proliferative capacity, express both H_1 and H_2 histamine receptors coupled to phosphatidylinositol hydrolysis and not to the adenylyl cyclase system. This characteristic is in agreement with our previous reports on the expression of histamine receptors with atypical coupling to transductional pathways in experimental [8, 22] and human breast carcinomas [14], as well as in the normal mammary gland of young virgin female rats [38]. Though the biological meaning of this unusual second messenger pathway for H_2 agonists remains to be clarified, the implication of cAMP in the regulation of growth in normal and malignant cells [39, 40], including breast cancer cells, must be emphasized [41]. Recent studies have linked cAMP negatively with the control of mitogenic signaling pathways, via modulation of MAP kinase [42, 43]. The fact that H_2 receptors have lost their ability to stimulate cAMP production, would imply the deprivation of a regulatory mechanism of cell growth. We would like to highlight the value of these cell lines exhibiting a high level of endogenous histamine receptors, in contrast with other models overexpressing an exogenous transgene. These cells represent a very interesting model to analyze the atypical linkages of histamine receptors found in different systems.

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