Histamine as a Growth Factor and Chemoattractant for Human Carcinoma and Melanoma Cells: Action through Ca^{2+}-Mobilizing H_1 Receptors

Ben C. Tilly,* Leon G. J. Tertoolen,* Rolf Remorie,* Annie Ladoux,‡ Ingrid Verlaan,‡ Siegfried W. de Laat,* and Wouter H. Moolenaar‡

*Hubrecht Laboratory, Netherlands Institute for Developmental Biology, 3584 CT Utrecht, the Netherlands, and the ‡Division of Cellular Biochemistry, the Netherlands Cancer Institute, 1066 CX Amsterdam, the Netherlands

Abstract. Histamine receptors are present on the surface of various normal and tumor-derived cell types, where their biological function is incompletely understood. Here we report that histamine not only stimulates cell proliferation under serum-free conditions, but also is chemotactic for human carcinoma (Hela and A431) and melanoma (A875) cells expressing H_1 type receptors. Histamine was found to be a potent activator of phospholipase C, leading to polyphosphoinositide hydrolysis and subsequent intracellular Ca^{2+} mobilization. In addition, histamine also causes the protein kinase C–mediated activation of Na^+/H^+ exchange, as evidenced by an amiloride-sensitive rise in cytoplasmic pH. All histamine-induced responses, including chemotaxis and DNA synthesis, are completely inhibited by the H_1 receptor antagonist pyrilamine, but not by cimetidine, an inhibitor of histamine H_2 type receptors. Our results suggest that histamine may have a previously unrecognized role in the migration and proliferation of cells expressing H_1 receptors.

HISTAMINE is a widely occurring chemical mediator that has long been known as a neurotransmitter, inflammatory factor, and a modulator of gastro-intestinal functions (10, 24). It has also been suggested that histamine is involved in certain types of cell proliferation in vivo, such as wound healing, embryonic development, and tumor growth (for review, see reference 2). There are several reports that elevated levels of histamine and of its synthesizing enzyme are associated with rapid tissue growth (17), but the precise link, if any, between histamine and cell proliferation remains obscure.

Three types of histamine receptors (H_1, H_2, and H_3) have been identified that differ in their sensitivity to antagonists and mediate different actions (14). While activation of H_2 type receptors leads to an increase in cAMP (12), the H_1 type receptor is linked to phosphoinositide breakdown and Ca^{2+} mobilization (8, 9, 23) and, therefore, is an attractive candidate for mediating growth stimulation.

Here we report that exogenously added histamine not only stimulates DNA synthesis and cell division but also evokes a chemotactic response in human Hela and A431 carcinoma cells and A875 melanoma cells. We show that these novel actions of histamine are mediated by the H_1 type receptor that triggers the hydrolysis of phosphoinositides with consequent formation of various second messengers. Our results suggest that histamine may have a novel role in the migration and proliferation of H_1 receptor–bearing (tumor) cells.

Materials and Methods

Materials

BSA, cimetidine, DiC8, histamine, and pyrilamine were purchased from Sigma Chemical Co. (St. Louis, MO). Other reagents were from the following sources: Bis(carboxy-ethyl)carboxyfluorescein (BCECF)[1] from Molecular Probes (Eugene, OR) epidermal growth factor (EGF), receptor grade, from Collaborative Research, Inc. (Waltham, MA); FCS from Hyclone Laboratories (Logan, UT); Hoechts 33258 from Boehringer Mannheim GmbH (Mannheim, FRG); indo-1 acetoxymethylester from Molecular Probes (Eugene, OR) and polycarbonate filters from Nuclepore Corp. (Pleasanton, CA). Myo[2-^{3}H]inositol (12.3 Ci/mmol) and cAMP assay kits were obtained from Amersham International (Amersham, UK).

Cell Culture

Human A431 and Hela carcinoma and A875 melanoma cells were routinely grown at 37°C in DME containing 7.5% (vol:vol) FCS at 5% CO_2.

Cell Counting

Hela, A431, and A875 cells were seeded at a density of $10^3/cm^2$ (Hela and A431) or $5 \cdot 10^2/cm^2$ (A875) in DME containing 7.5% (vol:vol) fetal calf serum and allowed to attach for 24 h. After shifting the cultures to serum-

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B. C. Tilly's present address is the Department of Biochemistry I, Medical Faculty, Erasmus University, Rotterdam, the Netherlands.

[1] *Abbreviations used in this paper*: BCECF, bis(carboxy-ethyl)carboxyfluorescein; EGF, epidermal growth factor; IMBX, isobutylmethylxanthine.

free DME/Ham's F-12 (1:1; vol:vol) medium, supplemented with 10 μg/ml transferrin, growth factors were added. 24–48 h later cells were resuspended and counted. Generation time (cell doubling time) was calculated from cell counts after 24 and 48 h of stimulation.

DNA Content

Cells were seeded and allowed to attach in DME. 24 h before growth factor addition, the cultures were shifted to serum-free medium consisting of DME/Ham's F-12 (1:1, vol:vol), supplemented with 10 μg/ml transferrin. After 2 d of hormonal stimulation, a cell lysate was prepared and total DNA content was measured using the nuclear dye Hoechst 33258 (18).

Determination of Inositol Phosphates

Nearly confluent cultures were shifted to serum-free DME/Ham's F-12 medium, containing 10 μg/ml transferrin and 2 μCi [^{3}H]inositol for 24 h. After stimulation, an inositol phosphate containing fraction was prepared and quantitated by anion exchange chromatography as described previously (27). HPLC elution profiles were obtained by analyzing inositol phosphates containing fractions on a Partisil Sax column, eluted with a gradient to 1.5 M ammonium formate/phosphoric acid (pH, 3.7). [^{3}H]radioactivity was determined by on-line scintillation counting using a radioactivity monitor (Berthold LB506C; Betrow, Rotterdam, the Netherlands).

cAMP Measurements

Nearly confluent cells in 6-well tissue culture dishes were preincubated for 2 h in serum-free DME containing 20 mM Hepes (pH, 7.6). Thereafter, cells were exposed to 1 mM isobutylmethylxanthine (IMBX), and, 10 min later, were stimulated with agonist. The reactions were stopped by adding 10% (wt/vol) ice-cold TCA. After centrifugation, the supernatants were extracted with diethylether to remove TCA and neutralized with Tris-base. Cellular content of cAMP was determined using the [^{3}H]cAMP assay kit from Amersham International according to the instructions of the manufacturer.

Ionic Responses

Nearly confluent cultures, attached to glass coverslips and maintained in serum-free DME/Ham's F-12 (1:1, vol:vol) medium containing 10 μg/ml transferrin for 24 h, were loaded with the fluorescent indicators indo-1 (for Ca^{2+}) or BCECF (for pH_i). Fluorescence was recorded and calibrated as described previously (21).

Chemotactic Assay

Chemotactic assays were carried out in modified Boyden chambers (11), equipped with gelatin-coated filters from Nuclepore Corp. (8 μm pore size). Cells, resuspended to a density of 10^6 cells/ml, were seeded into the upper chamber, while a histamine-containing agar solution (5%) was put in the lower chamber. All incubations were performed in DME/Ham's F-12 (1:1, vol:vol) medium containing 10 μg/ml transferrin and 1 mg/ml BSA. The results are expressed as number of cells migrated through the membrane per microscopic field (400×) during a 12-h period.

Results

Mitogenesis

In an initial screening, we found Ca^{2+}-mobilizing H_1 receptors to be present on various human tumor cell lines, including Hela, A431, epidermoid carcinoma, HT-29, colon carcinoma, and A875, melanoma cells, but not on MCF-7 breast carcinoma cells. No functional H_1 receptors were found on mouse NIH and Swiss 3T3 cells or on rat-1 fibroblasts. The Hela carcinoma cell line constitutes a convenient model for studying potential effects of histamine on cell proliferation, since these cells can be grown under serum-free conditions while they remain growth factor-responsive (15). When kept in a serum-free medium containing transferrin, Hela cells remain viable with a mean population doubling time of 72 h.

Table I. Growth Stimulation of Human Carcinoma and Melanoma Cells by Histamine

	Cell density after 48 h (cells × 10^{-2}/cm^2)			Mean generation time (*h*)		
	Hela	A431	A875	Hela	A431	A875
Control	16 ± 1	19 ± 1	10 ± 1	72	50	56
Histamine (100 μM)	26 ± 2	30 ± 2	16 ± 1	31	29	36
Insulin (5 μg/ml)	26 ± 2	28 ± 1	16 ± 1	31	30	36
EGF (50 ng/ml)	51 ± 2	ND	11 ± 1	19	ND	48

Cells were seeded and allowed to attach for 24 h before growth stimulation. 24–48 h after addition of the various growth factors, cells were resuspended and counted. Data are expressed as means ± SEM for triplicate experiments.

Addition of histamine to such serum-free cultures was found to stimulate DNA synthesis and cell division in a dose-dependent manner, half-maximal effects being observed at 10–15 μM and a saturating response at 5 × 10^{-5} M. As shown in Table I and Fig. 1 histamine is somewhat less potent as EGF in stimulating Hela cell growth, with histamine (10^{-4} M) decreasing generation time from 72 to 31 h and EGF (50 ng/ml) to 19 h. Insulin (5 μg/ml) was equally potent as histamine (Table I), whereas mitogenic peptides such as bombesin, bradykinin, and substance P, had no effect on Hela cell proliferation (our unpublished observations). When added together, histamine and insulin evoke an additive rather than a synergistic proliferative response (not shown). The mitogenic activity of histamine is not restricted to Hela cells. Also in A431 cells and A875 melanoma cells histamine exerts a marked mitogenic effect (Table I).

The growth stimulatory action of histamine appears to be mediated by the H_1 type receptor, since the H_1 antagonist pyrilamine completely blocks histamine-induced DNA synthesis, whereas the H_2 antagonist cimetidine, even at millimolar concentrations, has no detectable effect (Fig. 1). Importantly, these antagonists affect neither the basal rate nor the EGF- and/or insulin-stimulated rate of DNA synthesis (Fig. 1).

Involvement of Phospholipase C

As illustrated in Fig. 2, addition of histamine to Hela cells evokes the phospholipase C-mediated breakdown of polyphosphoinositides which results in rapid formation of inositol

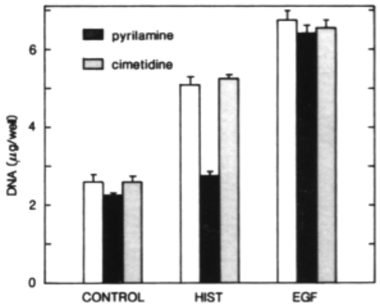

Figure 1. Effects of histamine, EGF and histamine receptor antagonists on cellular DNA content in Hela cells. Concentrations used: histamine, 100 μM; EGF, 50 ng/ml; pyrilamine, 1 μM; cimetidine, 1 mM. Values are given as means ± SEM for triplicate cultures. Qualitatively similar results were obtained with A4311 and A875 cells.

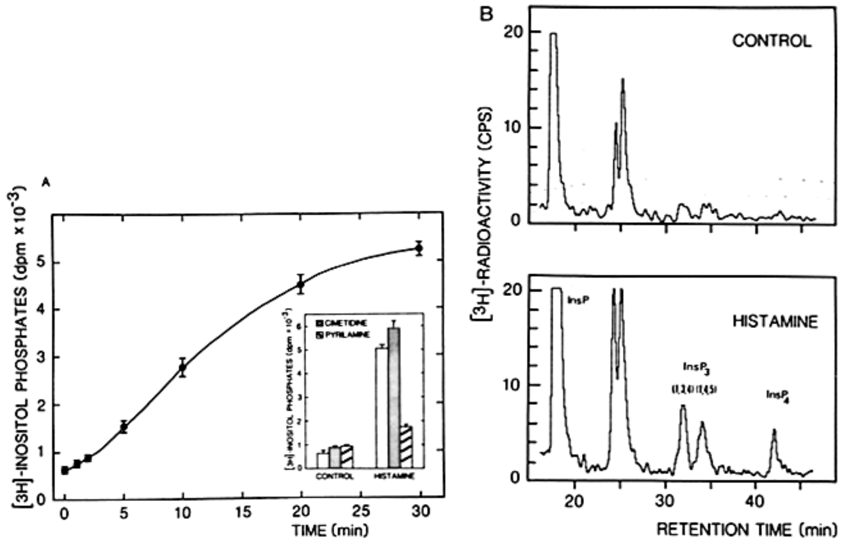

Figure 2. Accumulation of inositol phosphates evoked by histamine in Hela cells. *A*, time course of histamine-induced inositol phosphate accumulation in Li$^+$-treated cultures. [^{3}H]-Inositol prelabeled cultures were incubated with histamine (100 μM) for the indicated times in the presence of 10 mM LiCl. Thereafter, an inositol phosphate containing fraction was prepared and analyzed as described under Materials and Methods. Inset shows the effects of pyrilamine (1 μM, hatched bars and cimetidine; 1 mM, dotted bars) on the histamine-induced inositol phosphate production (30-min incubations). Data are expressed as means ± SEM for triplicate incubations. *B*, HPLC profiles of [^{3}H]inositol phosphates obtained from control and histamine-stimulated cultures (100 μM during 10 s).

phosphates (Fig. 2 *A*). Half maximal stimulation of inositol phosphate formation is observed at approx. 1–2 μM histamine, while the response saturates at 10 μM (Tilly, B. C., L. G. J. Tertoolen, A. C. Lambrechts, R. Remorie, S. W. de Laat, and W. H. Moolenaar, submitted for publication), which is in the same range of histamine-induced cell proliferation. Like the stimulation of DNA synthesis, inositol phosphate formation is inhibited by pyrilamine (IC_{50} ~15 nM at 100 μM histamine) but not by cimetidine (Fig. 2 *A*, *inset*), indicating the involvement of H_1 receptors. EGF and insulin had little or no effect on inositol phosphate levels in Hela cells (not shown), although other cell types, including A431 cells, do show an inositol phosphate response to EGF (13, 16, 28).

Analysis of the various inositol phosphates formed (Fig. 2 *B*), reveals a rapid, several-fold increase in Ins(1,4,5)P_3, the second messenger for Ca^{2+} mobilization (5), and other inositol polyphosphates. In addition to generating inositol phosphates, histamine transiently stimulates the formation of diacylglycerol in [2-^{3}H]glycerol-labeled cells, as revealed by using conventional TLC (Tilly, B. C., L. G. J. Tertoolen, A. C. Lambrechts, R. Remorie, S. W. de Laat, and W. H. Moolenaar, manuscript submitted for publication), which binds to and directly activates protein kinase C.

Ionic Responses

The activation of phospholipase C by histamine is expected to lead to increases in cytoplasmic free Ca^{2+} ($[Ca^{2+}]_i$) and pH (pH_i), because of Ins(1,4,5)P_3-mediated Ca^{2+} release (5) and protein kinase C–activated Na^+/H^+ exchange (20), respectively. Fig. 3 shows typical ionic responses to histamine. Histamine elicits a rapid, but transient, biphasic rise in $[Ca^{2+}]_i$, which approaches micromolar Ca^{2+} levels at 10–20 s after hormone addition and which is completely blocked by pyrilamine (Fig. 3 *A*). The initial rise in $[Ca^{2+}]_i$ rise is largely, if not entirely, because of release of intracellular stored Ca^{2+}, while the second phase requires Ca^{2+} influx across the plasma membrane (Tilly, B. C., L. G. J. Tertoolen, A. C. Lambrechts, R. Remorie, S. W. de Laat, and W. H. Moolenaar, manuscript in preparation).

Histamine also causes the protein kinase C–mediated activation of Na^+/H^+ exchange, as shown by a rise in pH_i of ~0.20 U, that is abolished in the presence of the Na^+/H^+ exchange inhibitor amiloride (0.5 mM), while subsequent addition of cell permeable diacylglycerol (diC8) does not cause an additional elevation of pH_i (Fig. 3 *B*). Similar ionic responses to histamine were observed in A431 and A875 cells.

Independence of Adenylate Cyclase

Although the mitogenic effects of Ca^{2+} mobilizing hormones are thought to proceed via the phospholipase C signal transduction pathway, recent evidence suggests that inhibition of adenylate cyclase (through a receptor-linked inhibitory G protein; G_i) is actually more important for mitogenesis (26). We determined the effects of histamine on

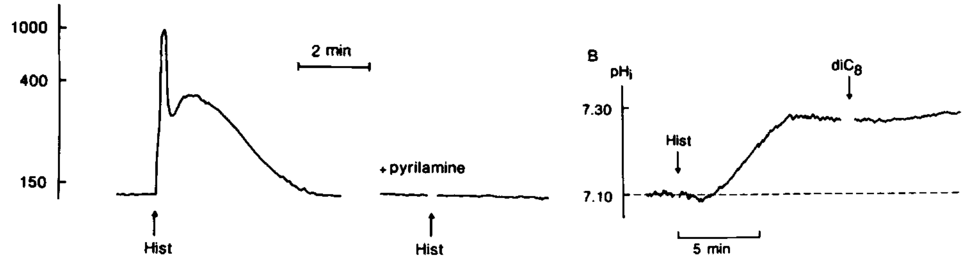

Figure 3. Histamine-induced ionic responses in Hela cells. *A*, increase in $[Ca^{2+}]_i$ after addition of histamine (100 μM) in the absence and presence of pyrilamine (1 μM), as indicated. *B*, rise in cytoplasmic pH (pH_i) induced by histamine; no further increase is observed after subsequent addition of dioctanoyl (diC8, 50 μM).

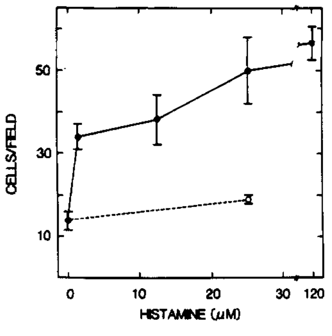

Figure 4. Induction of a chemotactic response by histamine in Hela cells and inhibition by pyrilamine (250 nM). Chemotactic assays were performed as described under Materials and Methods. The indicated histamine concentration represents the final concentration after complete equilibration of both upper and lower chamber. Data are expressed as the number of cells ± SEM ($n = 3$) migration through the membrane per microscopic field (400×).

intracellular levels of adenosine 3′,5′-monophosphate (cAMP) following stimulation of adenylate cyclase by isoproterenol (10 μM); the phosphodiesterase inhibitor IMBX (0.5 mM) was included to ensure that changes in cAMP levels were not mediated by phosphodiesterase action. Under conditions where exogenous phosphatidic acid (cf. reference 22) inhibits cAMP accumulation up to 60% within 15 min (from 1.23 to 0.30 ± 0.04 pmol cAMP/10^6 cells), histamine completely failed to attenuate isoproterenol-induced cAMP accumulation in Hela cells. Thus, mitogenic signaling through the H_1 type receptor does not appear to involve the G_i protein that inhibits adenylate cyclase.

Chemotaxis

Several agents, including certain growth regulators, promote the chemotactic migration of their target cells (4, 25, 29). This activity is important in wound healing, but chemotaxis may also be involved in tumor cell metastasis (19). We examined whether histamine can function as a chemoattractant for Hela cells using the modified Boyden chamber assay (11), in which cells are allowed to migrate through membrane filters. Fig. 4 shows that histamine evokes a potent chemotactic response in a dose-dependent manner, with a half-maximal effect at $\sim 5 \times 10^{-6}$ M. Histamine-induced chemotaxis is completely blocked by pyrilamine (Fig. 4), indicating again the involvement of H_1 receptors. A chemotactic response (an approximately twofold increase relative to the control) is also observed after stimulating A875 melanoma cells with histamine (100 μM), although the chemotaxis assay was technically more difficult than with Hela cells because of the tendency of melanoma cells to aggregate when resuspended in a Boyden chamber.

Discussion

The present data clearly demonstrate that histamine, a ubiquitously occurring local hormone, can function as a growth factor and chemoattractant for cells types that express the Ca^{2+}-mobilizing H_1 type receptor. We found no evidence that mitogenic signaling through H_1 type receptors may involve alterations in cAMP levels. Our results raise the intriguing possibility of a role for histamine in promoting cell growth and migration in vivo. The present findings also suggest that histamine-secreting mast cells may have a more important role in modulating cell proliferation than has been assured to date. Indeed, the finding that mast cells are frequently abundant in the contiguous tissue of metastatic tumors supports this possibility (7).

Interestingly, histamine is not only stored in mast cells but is also actively synthesized in various other cell types. In particular, high levels of histamine and its synthesizing enzyme occur in many tissues undergoing rapid cell growth or tissue repair, including regenerating liver, bone marrow, embryonic tissues, and experimental tumors (1, 10, 17, 6). Although it remains to be seen whether histamine produced by these cells is released into the extracellular space, it is tempting to speculate that newly synthesized histamine, acting via H_1 receptors, may contribute to cell proliferation and development by serving as an autocrine growth and motility factor.

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