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EXPRESSION OF HISTAMINE H_1 RECEPTORS ON CULTURED HISTIOCYTIC LYMPHOMA CELLS*

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Abstract—Histamine H_1 receptors were identified in U937 human histiocytic lymphoma cells using a radioligand binding technique with [^{3}H]mepyramine. Reversible high-affinity binding with this ligand was obtained, and specificity of binding for selected H_1 agonists and antagonists was demonstrated. Competition binding experiments with mepyramine and histamine yielded results consistent with single-site binding for mepyramine and two-site binding for histamine. Dissociation constants for the high- and low-affinity histamine binding states were 6.8×10^{-6} and 2.4×10^{-4} M respectively. The high-affinity state for histamine binding was abolished when the membranes were coincubated with 100 μM guanosine-5′-*O*-(3-thiotriphosphate) (GTPγS). Saturation binding studies yielded an average of 66 fmol/mg protein binding sites (6000 receptors per cell) with a [^{3}H]mepyramine K_D of 9.6 nM. When differentiation of these cells was induced by phorbol-myristate-acetate, receptor density increased by 73% to 114 fmol/mg protein. This increase in receptor density was inhibited by actinomycin D and cycloheximide. Exposure of native and differentiated U937 cells to 10^{-5} and 10^{-4} M histamine for 24 hr resulted in a dose-dependent down-regulation in receptor density. The data indicate that U937 cells may provide a model cell line for the study of histamine receptor gene expression.

Histamine is an important autocoid which is known to mediate inflammation. Histamine exerts its effects through histamine H_1 and H_2 receptors which are located on cell plasma membranes [1, 2]. H_3 receptors have also been described [3]. Histamine H_1 receptors have been identified on lymphocytes and monocytes [4, 5]. Activation of histamine H_1 receptors stimulates the generation of superoxide anions and chemiluminescence, stimulates complement component C3 gene expression and C3 biosynthesis, and augments interferon-γ induced phagocytosis in macrophages [6–8].

The study of histamine H_1 receptor function and regulation has been hampered by inconsistencies and low specific binding in the radioligand binding technique most commonly used to identify the receptor. In general, the application of radioligand binding with [^{3}H]mepyramine has yielded more consistent results in tissues that are relatively homogenous [9]. In an elegant series of experiments, Nakahata and co-workers [10] identified and characterized histamine H_1 receptors in cultured human astrocytoma cells. Radioligand binding with [^{3}H]mepyramine correlated with biochemical responses to histamine in these cells.

In the present study, we identified and characterized histamine H_1 receptors in a monoblastic cell line (U937) with a known potential to differentiate into a cell with properties of a mature macrophage. This cell line was derived from a patient with histiocytic lymphoma [11]. The cells bear markers characteristic of promonocytes and they differentiate into macrophage-like cells upon stimulation with phorbol esters, interferon-γ, and lymphokine [12, 13]. Plasma membrane receptors for immunoglobulin Fc, fmet-leu-phe and complement are expressed in these cells in the differentiated state [13, 14]. U937 cells were found to possess membrane-associated histamine H_1 receptors. Treatment of these cells with phorbol-12-myristate-13-acetate (PMA) was associated with a protein synthesis-dependent increase in histamine receptor density. Dose-dependent down-regulation of histamine receptors was observed in native and PMA-treated cells exposed to histamine.

MATERIALS AND METHODS

Materials. The U937 cell line was obtained through the American Type Culture Collection, (Rockville, MD). RPMI-1640, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), penicillin/streptomycin, Trypan blue stain, L-glutamine, and heat-inactivated fetal calf serum (FCS) were all purchased from the Grand Island Biological Co. (Grand Island, NY). [^{3}H]Mepyramine (26 Ci/mmol), [^{3}H]histamine (47 Ci/mmol), and [^{3}H]leucine (57 Ci/mmol) were obtained from the Amersham Corp. (Arlington Heights, IL). 2-Methylhistamine, 4-methylhistamine, and metiamide were gifts from Smith, Kline, & French Laboratories, (Philadelphia, PA). Stereoisomers of chlorpheniramine were gifts from the Schering Corp. (Bloomfield, NJ). Tris and guanosine-5′-*O*-(3-thiotriphosphate) (GTPγS) were obtained from Boehringer Mannheim (Indianapolis, IN). All other chemicals were obtained from the Sigma Chemical Co. (St Louis, MO). Non-radioactive mepyramine was obtained as the maleate salt.

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Methods. The description and characterization of the U937 cell line, derived from a patient with histiocytic lymphoma, have been reported [11]. The cells were grown in RPMI-1640 supplemented with 15% FCS, 10 mM HEPES, 2 mM L-glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin in a 5% CO_2 humidified atmosphere. Cellular density was maintained between 0.3×10^6 cells/ml and 1.5×10^6 cells/ml with twice weekly passages. Mortality did not exceed 15% during any experimental condition. The viability of the cells was ascertained using Trypan blue exclusion.

Cell numbers were determined with a hemocytometer prior to the addition of PMA. PMA was dissolved in 1 cc of ethanol in sufficient quantity to provide a concentration of 5×10^{-8} M in the culture medium. Quantitation of cell growth and adherence were performed as described by Ways *et al.* [15]. Undifferentiated cells were round and non-adherent. Addition of PMA to the cell suspension resulted in a dramatic change in cell morphology that was evident at 24 hr. PMA-treated cells were densely clumped, and 38% was adherent to plastic (one determination). Cell growth was also inhibited (two determinations). PMA treatment has been found previously to inhibit the growth of U937 cells [13, 15], and adherence to plastic surfaces has been found to represent the expression of a more differentiated phenotype [15, 16]. Owing to difficulties in the routine counting of clumped and adherent cells, cell numbers were determined prior to PMA treatment. Each experimental treatment was performed using 200×10^{-6} cells, and each group of cells was treated, processed, and analyzed separately.

The effects of PMA on histamine H_1 receptor induction were investigated after 4- and 24-hr exposure periods. The effect of protein synthesis inhibition on receptor expression was investigated by co-incubation with either 1 μg/ml cycloheximide or 0.01 μg/ml actinomycin D along with PMA for 24 hr. Protein synthesis inhibition was confirmed by incubating 30×10^6 cells in 20 ml of medium containing 5 μCi of [^{3}H]leucine in the presence or absence of cycloheximide (1 μg/ml). Cycloheximide was added to the medium 30 min prior to the addition of [^{3}H]leucine. Following incubation, the cells were washed and resuspended in 1.5 ml of Hanks' balanced salt solution. Two 0.5-ml aliquots were analyzed for [^{3}H]leucine incorporation by denaturing the cells with 0.5 ml of 1 N NaOH. Protein was then precipitated by adding 5 ml of cold 25% trichloroacetic acid (TCA). The solution was vortexed and chilled on ice for 30 min. The protein was collected by suction filtration and washed with 15 ml of 5% TCA. Radioactivity on the filters was measured by counting in a scintillation counter. The protein content of the third aliquot of cells was determined according to the method of Lowry *et al.* [17], using bovine serum albumin as a standard.

Prior to all treatments, cells were placed in fresh medium. Cells were seeded onto petri dishes at a concentration of 1.0 to 1.5×10^6 cells/ml and incubated for 24 hr. Following the treatment, cells were harvested by scraping with a rubber policeman. The cells were collected by centrifugation at 250 *g* for 10 min and resuspended in Tris buffer in preparation for radioligand binding.

Radioligand binding. Binding experiments were performed as previously described [9]. Crude membrane preparations rather than whole cells were used for binding experiments. We were unable to successfully obtain specific [^{3}H]mepyramine binding with whole cells. In addition, the cell clumping associated with PMA treatment would be expected to prevent access of free [^{3}H]mepyramine to many of the binding sites. Membranes were prepared for incubation by the addition of 5 ml of cold buffered saline (75 mM Tris, 150 mM NaCl, 1.5 mM $CaCl_2$, pH 8.0, at 4°). The cells were disrupted by Polytron action for 25 sec. Buffered saline was used to wash the membranes into a chilled Sorvall tube which was then centrifuged for 20 min at 41,000 *g*. The pellet was resuspended in a minimum volume of buffered saline (4–8 ml) with a Teflon pestle homogenizer (5 strokes). For time course experiments, 100 μl of the membrane mixture was added to duplicate test tubes containing 25 μl of [^{3}H]mepyramine and 25 μl of buffered saline. Membranes were incubated at 37° for various lengths of time. The final composition of the incubation buffer was 62.5 mM Tris, 125 mM NaCl, 1.25 mM $CaCl_2$, and 1 nM [^{3}H]mepyramine at pH 7.5. Incubation was terminated by rapid filtration over Whatman GF/C filters (Whatman, Inc., Clifton, NJ). Test tubes and filters were washed quickly with 25 ml of cold buffered saline. After 10 min of incubation, 25 μl of 6×10^{-2} M histamine dihydrochloride (with pH adjusted to 7.5) was added to duplicate tubes, and the membranes were filtered after additional incubation.

For competition experiments, 100 μl of the membrane preparation was added to triplicate test tubes containing 25 μl of 6–8 nM [^{3}H]mepyramine and 25 μl of buffered saline with various concentrations of the specified agonists and antagonists. The final concentration of [^{3}H]mepyramine in these experiments was 1.0 to 1.3 nM. Histamine and its derivatives were used from a concentration of 10^{-7} M up to a final concentration of 10^{-2} M. Antagonists were used in concentrations from 10^{-9} M to 10^{-3} M. Incubation was terminated as previously described after 20 min of incubation. For saturation experiments, 100 μl of membrane was added to triplicate test tubes containing either 25 μl of buffer or 6×10^{-2} M histamine and 25 μl of [^{3}H]mepyramine (final concentration 2.0 to 33 nM). Filters were placed in scintillation fluid and counted at about 50% efficiency. Protein analysis was performed as previously described. Protein concentrations used in binding experiments ranged from 1.0 to 1.6 mg/ml.

Non-specific binding was defined as the amount of [^{3}H]mepyramine bound in the presence of 10^{-2} M histamine. While it should be acknowledged that this is a very high concentration, inhibition of [^{3}H]mepyramine binding by 10^{-2} M histamine was generally equivalent to that induced by 10^{-6} M non-radioactive mepyramine. With an observed K_D of 5–10 nM, a mepyramine concentration of 10^{-6} M would provide maximal antagonism of histamine. Specific binding was usually 60–80% of total binding, whether defined by 10^{-2} M histamine or 10^{-6} M mepyramine. Saturation curves were used to determine receptor density and [^{3}H]mepyramine dissociation constants.

Competition curves were used to determine agonist and antagonist dissociation constants (K_D) and slope factors.

Receptor down-regulation. Histamine receptor down-regulation was induced by incubation of U937 cells with 10^{-5} and 10^{-4} M histamine dihydrochloride in RPMI-1640. In these experiments, U937 cells were stimulated to differentiate by incubating the cells with PMA for 24 hr. The cells were washed twice with fresh medium and then incubated with medium containing either 10^{-5} or 10^{-4} M histamine dihydrochloride for an additional 24 hr. The cells were washed twice with 10 ml of Tris buffer prior to membrane preparation for receptor assys.

The metabolic fate of down-regulated receptors was examined by incubating desensitized cells in fresh medium with cycloheximide (1 μg/ml). Crude membrane preparations were obtained as previously described, and the supernatant fractions were subjected to high speed centrifugation fractions (100,000 *g*) for 1 hr to determine whether [^{3}H]pyrilamine binding was present in the sediment obtained from this fraction.

To determine whether reduced receptor density was an artifact of inadequate histamine washout, 2.5 μCi of [^{3}H]histamine was added as a tracer to 15×10^6 cells in 10 ml of medium containing 10^{-5} M histamine. The cells were incubated for 24 hr, membranes were prepared as described above, and the radioactivity in the membrane preparation was determined by scintillation counting.

Curve modeling and statistics. Data from individual competition and saturation experiments were analyzed using computer modeling methods as previously described [9]. Data points obtained from each group of 200 million cells were analyzed separately. Slope factors were derived by fitting competition data with a 4 parameter logistic equation using ALLFIT [18]. The dissociation constants and receptor densities were derived by applying the law of mass action to the experimentally-derived binding data using EBDA and LIGAND [19, 20]. Determination of the binding states of the receptor for histamine and histamine antagonists was made by fitting the binding data to a one- or two-site receptor model (LIGAND). Separate fits of the data were compared by an F test to search for improvement in the goodness-of-fit for a two-site versus a one-site model. A two-site model was accepted only if the fit was significantly better than a one-site model ($P < 0.05$). Statistical comparisons between groups of experiments were made using Student's *t*-test [21]. Receptor densities and dissociation constants were determined for saturation experiments using the LIGAND program. Scatchard plots were also constructed for each experiment. The receptor densities and dissociation constants derived by linear regression were nearly identical to those obtained with LIGAND.

RESULTS

Time course. A representative time course experiment is illustrated in Fig. 1. [^{3}H]Mepyramine binding to the cell membranes was very rapid, and a very high level of binding was initially achieved, followed by a more gradual dissociation phase until equilibrium was reached at 6–10 min. After 10 min of incubation, addition of excess histamine resulted in displacement of [^{3}H]mepyramine from the membrane-binding sites. The average specific binding for these three experiments was 65%. The nature of the initial high level of binding was investigated by performing time course experiments in a variant U937 cell line grown for a prolonged period in 5% FCS. We have found that U937 cells lose all specific [^{3}H]mepyramine binding when grown continuously for 4–6 months. An identical initial high level of binding was observed in crude membranes from these cells. Thus, the initial high level of binding appeared to be a membrane associated, non-receptor phenomenon.

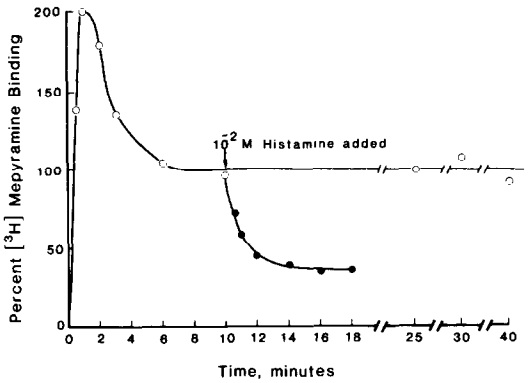

Fig. 1. Time course of the binding of [^{3}H]mepyramine to U937 cell membranes. Membranes were incubated as described under Materials and Methods. The abscissa represents the time of incubation. The ordinate represents the percent of maximal [^{3}H]mepyramine binding to the membrane preparation at equilibrium. After 10 min of incubation, sufficient histamine was added to the incubation mixture of certain tubes to obtain a concentration of 10^{-2} M. Data points are an average of two determinations. Incubation was terminated by rapid filtration at each time point noted. Data points indicate histamine-free (○) or histamine added (●) incubation conditions.

Saturation experiments. The results of a representative saturation are illustrated in Fig. 2A. Nonspecific binding was linearly related to the [^{3}H]mepyramine concentration. A representative plot of bound/free versus specifically bound [^{3}H]mepyramine is illustrated in Fig. 2B. The saturation curves and Scatchard plot were consistent with non-cooperative binding of [^{3}H]mepyramine to a single membrane-binding site.

The effects of various treatments on histamine receptor densities are illustrated in Fig. 3. The average histamine receptor density in native U937 cells was 66 ± 8 (SE) fmol binding sites/mg of protein (eight experiments). The average K_D for [^{3}H]-mepyramine was 9.6 ± 1.5 nM. Twenty-four-hour exposure of the cells to 5×10^{-8} M PMA resulted in a 73% increase in receptor density to 114 ± 9 fmol/mg protein (thirteen experiments). The difference in receptor densities was significant at the $P < 0.01$ level. The average K_D for [^{3}H]mepyramine obtained in these experiments was 7.6 ± 1.1 nM.

Receptor density was not altered after a 4-hr

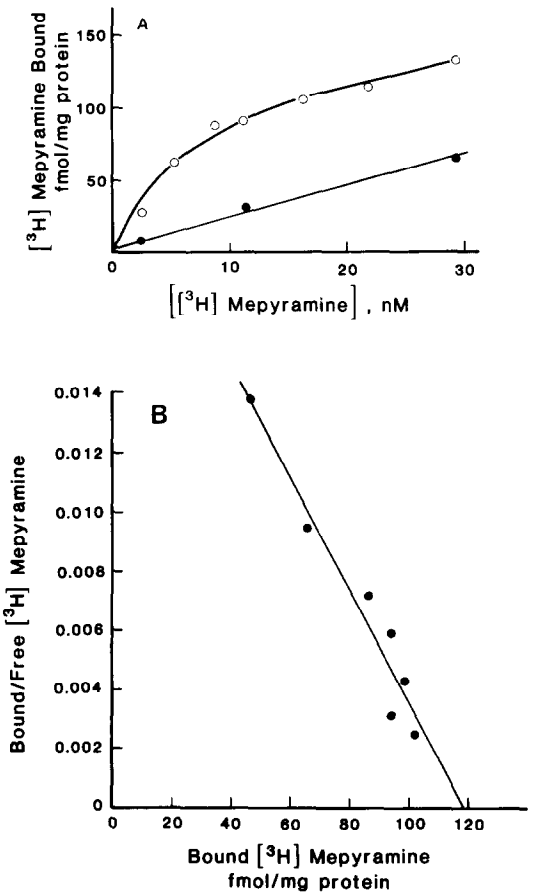

Fig. 2. (A) Saturation data for binding of [^{3}H]mepyramine to U937 cell membranes. The abscissa represents the concentration of [^{3}H]mepyramine in the incubation mixture. The ordinate represents the concentration of [^{3}H]mepyramine bound to membranes. Data points indicate total [^{3}H]mepyramine binding (○) and non-specific [^{3}H]mepyramine binding (●). All data points are the mean of triplicate determinations. The curves shown represent the computer-generated best fits according to the law of mass action, as described in Materials and Methods. (B) Scatchard plot of data shown in Fig. 2A. The abscissa represents the concentration of specifically bound [^{3}H]mepyramine. The ordinate represents the ratio of specifically bound [^{3}H]mepyramine to free [^{3}H]mepyramine in the incubation mixture.

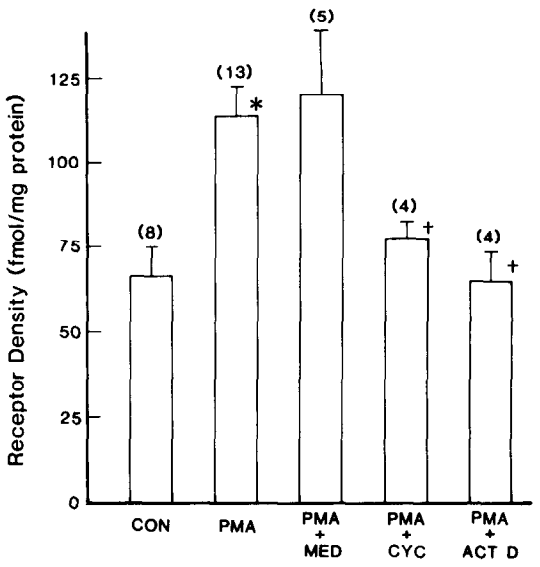

Fig. 3. Effects of various treatments on histamine H_1 receptor density in U937 cells. The ordinate represents the receptor density expressed in femtomoles per milligram of protein. The different experimental conditions are expressed on the abscissa. CON denotes native undifferentiated cells; PMA denotes differentiated cells after 24-hr treatment with PMA; PMA + MED denotes cells treated with PMA for 24 hr followed by additional incubation for 24 hr in fresh medium; PMA + CYC denotes cells treated with PMA and cycloheximide for 24 hr; and PMA + ACT D denotes cells treated with PMA and actinomycin D for 24 hr. Values are means ± SE; the number of experiments for each treatment is given in parentheses. Comparison of PMA-treated cells with native cells yielded differences significant at $P < 0.01$ (*). Comparison of PMA and cycloheximide and PMA and actinomycin D with PMA-treated cells yielded differences significant at $P < 0.05$ (+).

exposure to PMA. Removal of PMA from the medium after 24 hr, followed by additional incubation of the cells for 24 hr, resulted in a slight further increase in receptor density to 121 ± 18 fmol/mg protein (five experiments). The average K_D for [^{3}H]mepyramine obtained in these experiments was 8.6 ± 1.2 nM. Forty-eight hours of exposure to PMA resulted in considerably higher cell mortality. Separation of viable adherent cells from non-viable non-adherent cells revealed that the higher receptor densities were preserved in viable adherent cells after 48 hr of continuous PMA exposure.

Co-incubation of cells with PMA and either 1.0 μg/ml cycloheximide or 0.01 μg/ml actinomycin D inhibited the effect of PMA on cell differentiation and, likewise, receptor density was not increased when compared with control cells. Cycloheximide used at this concentration inhibited 87% of [^{3}H]leucine incorporation into protein after 2, 4, and 24 hr of incubation. Comparison of receptor densities with 24-hr PMA treatment alone and PMA treatment along with cycloheximide or actinomycin D yielded differences that were significant at the $P < 0.05$ level. Since receptor densities were determined as femtomoles of binding sites per milligram of protein, the effects of PMA, cycloheximide, and actinomycin D on total cell protein content were investigated. The results are illustrated in Fig. 4. Protein content per cell in this series of experiments refers to the total cell protein content rather than protein recovered by high speed centrifugation. No significant differences were observed in the protein content per cell for any experimental condition. Derivation of receptor number per cell yielded 6,000 receptors per native cell, 12,000 receptors per PMA treated cell, 6,700 receptors per PMA and cycloheximide treated cell, and 6,060 receptors per PMA and actinomycin D treated cell.

Treatment of U937 cells with 1 μg/ml of cycloheximide for 2, 4, and 24 hr resulted in 87% inhibition of [^{3}H]leucine incorporation into protein (two experiments).

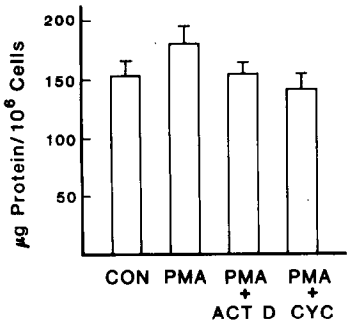

Fig. 4. Protein content of U937 cells. The ordinate represents micrograms of protein per million cells while the abscissa denotes various cell treatments. CON represents undifferentiated native cells, PMA represents cells treated with PMA for 24 hr, and PMA and ACT D and PMA and CYC denote cells treated for 24 hr with PMA and either actinomycin D or cycloheximide. Results are the mean of eight experiments for each condition ± SE.

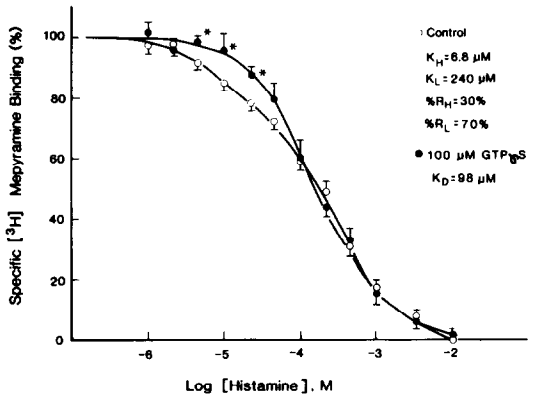

Fig. 5. Comparison of composite histamine competition curves with and without GTPγS. The abscissa represents the concentration of histamine and the ordinate represents the percentage of maximal specific [^{3}H]mepyramine binding. Data points are the means ± SE of nine experiments for histamine and seven experiments for histamine with GTPγS, each performed in triplicate. Comparison of data points at the concentrations of histamine denoted by an asterisk yielded differences that were significant at $P < 0.05$. The computer-drawn lines represent the best fit to the data with a two-site binding model for histamine and a one-site binding model for histamine with GTPγS.

Competition studies. Competition experiments were performed on cells treated with PMA for 24 hr. The competition curve for histamine is illustrated in Fig. 5. Analysis of individual curves for histamine with the LIGAND computer program yielded results that were consistent with two-site binding in six out of nine experiments. The average K_H (dissociation constant for the high-affinity state) was $6.8 \pm 2.3 \times 10^{-6}$ M, while the average K_L (dissociation constant for the low-affinity state) was $2.4 \pm 0.7 \times 10^{-4}$ M and the average % R_H (percent of receptors in the high-affinity state) was 30 ± 5. The average slope factor for these experiments was 0.67 ± 0.05. This slope factor is consistent with multisite binding for histamine. Co-incubation of the cell membrane preparation with 100 μM GTPγS resulted in the disappearance of the high-affinity binding state in each of seven experiments. These results are illustrated in Fig. 5. Analysis of these seven curves by computer modeling methods yielded only a single affinity state with a K_D of 9.8 ± 10^{-5} M. The average slope factor for these experiments was 0.98 ± 0.08. This slope factor is consistent with single-site binding in the presence of GTPγS. Comparison of each of the three points defining the high-affinity portion of the competition curve yielded differences between the histamine and histamine with GTPγS curves that were significant at the $P < 0.05$ level.

A representative mepyramine competition curve is illustrated in Fig. 6. Analysis of five competition curves for nonradioactive mepyramine by computer modeling methods yielded results consistent with single-site binding in four out of five experiments. The average K_D obtained in these experiments was 11.3 ± 5.5 nM. The average slope factor for these experiments was 0.92 ± 0.1, not significantly different from 1.0. This slope factor is consistent with single-site binding for mepyramine.

The results of competition curves for other histamine agonists and antagonists are given in Table 1. The results indicate that only selective H_1 agonists and antagonists displace [^{3}H]mepyramine from its membrane binding site at low concentrations relative to selective H_2 agonists and antagonists. Large differences were also noted between the dissociation constants for (+)- and (−)-chlorpheniramine. The slope factors for diphenhydramine and the chlorpheniramine stereoisomers were close to unity.

Receptor down-regulation. Treatment of U937 cells with histamine was performed in order to investigate the effects of histamine on regulation of receptor expression. The results are illustrated in Fig. 7. Native cells and PMA-treated cells were incubated with 10^{-5} and 10^{-4} M histamine for 24 hr. Addition of histamine to the medium resulted in a dose-dependent reduction in receptor density by 35% and 58% for native cells and 33% and 40% for PMA-treated cells respectively. Comparison of treatment receptor densities observed with 10^{-4} M histamine treatment and those obtained in native and PMA-treated cells yielded differences that were significant at $P < 0.05$ in both instances. Concentrations of histamine greater than 10^{-4} M were associated with high cell mortality (>15%). Histamine itself had no effect on the morphologic appearance of either native or PMA-treated cells. No significant change in the [^{3}H]mepyramine K_D was observed with histamine treatment.

Since histamine uptake and membrane incorporation has been described in HL-60 cells [22], a promyelocytic leukemia cell line, a separate series of experiments was performed to determine the adequacy of washing histamine from the U937 cells. The results of three experiments performed in duplicate indicated that a 130-fold dilution of [^{3}H]histamine occurred during the membrane purification steps. According to the results of histamine competition experiments, the concentrations of histamine retained in the receptor down-regulated membrane preparation ($<10^{-6}$ M) would be

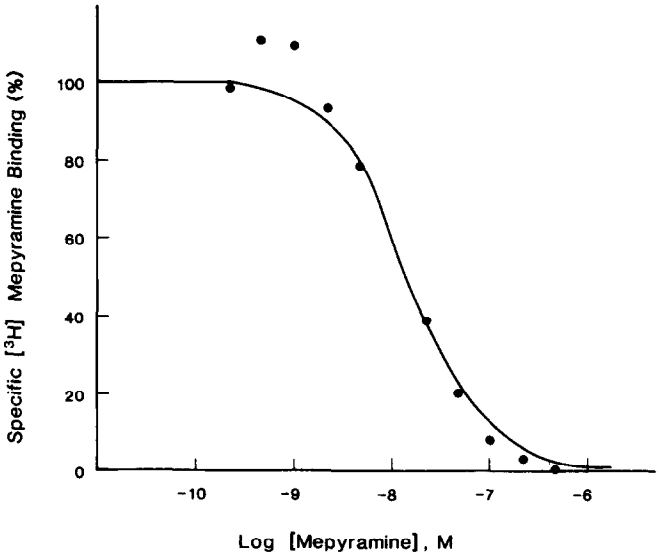

Fig. 6. Representative competition curve for non-radioactive mepyramine. The abscissa represents the concentration of mepyramine and the ordinate represents the percentage of maximal [^{3}H]mepyramine binding. Data points are the average of triplicate determinations. The computer-drawn line represents the best fit to the data with a one-site binding model.

Table 1. Binding parameters derived from [^{3}H]mepyramine competition curves for selected histamine agonists and antagonists

Agonist	N	K_D ($\times 10^4$ M)	Slope factor
2-Methylhistamine	4	3.3 ± 0.6	0.98 ± 0.19
4-Methylhistamine	4	>10	
Antagonist	**N**	**K_D ($\times 10^9$ M)**	**Slope factor**
Diphenhydramine	3	720 ± 160	1.13 ± 0.23
(+)-Chlorpheniramine	3	9.8 ± 1.8	0.97 ± 0.10
(−)-Chlorpheniramine	3	5500 ± 3000	0.95 ± 0.04
Cimetidine	4	>10^6	
Metiamide	3	>10^6	

Values are means ± SE.

expected to have a minimal effect on the binding displacement of [^{3}H]mepyramine. The low receptor densities observed in histamine-treated cells thus cannot be explained by inadequate washout of histamine. These observations are therefore consistent with histamine-induced receptor down-regulation.

The fate of down-regulated receptors of control cells treated for 24 hr with 10^{-4} M histamine was examined by washing the cells and incubating them in fresh medium with cycloheximide. After an additional incubation for 24 hr, receptor density increased from 28 ± 3 to 75 ± 4 fmol/mg protein (four experiments), a level not significantly different from control cells. [^{3}H]Mepyramine binding could not be detected in the supernatant fractions of the crude membrane preparation from either control cells or down-regulated cells (two experiments each).

DISCUSSION

The existence of histamine H_1 receptors on U937 cell plasma membranes is indicated by the presence of high-affinity reversible binding with [^{3}H]mepyramine. Three time course experiments demonstrated an initial high degree of binding followed by achievement of equilibrium binding after 5–6 min of incubation. The initial high level of binding appeared to be a non-receptor, membrane-associated phenomenon since it was also observed to occur in a variant cell line without identifiable receptors.

Saturation experiments yielded linear Scatchard plots in all experiments. This is consistent with single-site, non-cooperative binding of [^{3}H]mepyramine to the receptor. Single-site binding for mepyramine was also indicated by the steep slope of the mepyramine competition curves. The average slope factor for these experiments was close to unity. When competition curves were analyzed for multi-site binding using the LIGAND program, the results were consistent with single-site binding in four out of five experiments. Single-site binding for mepyramine and a mepyramine derivative has been reported in recent

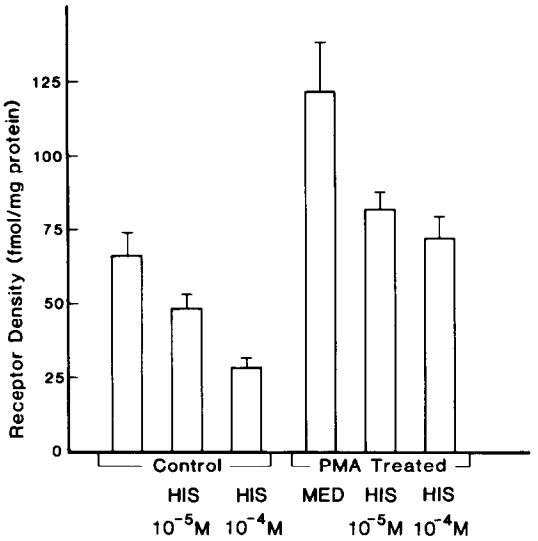

Fig. 7. Histamine-induced down-regulation of U937 cells. The ordinate represents histamine H_1 receptor density expressed as the percentage of receptors from cells not exposed to histamine. The abscissa denotes the various treatments. CON represents native undifferentiated cells. HIS represents native cells with histamine exposure. PMA + MED represent cells treated with PMA for 24 hr followed by incubation in medium for 24 hr. PMA + HIS represent cells treated with PMA for 24 hr followed by histamine exposure for an additional 24 hr. Values are means ± SE, N = 4. Comparison of CON cells with HIS, 10^{-4} M, and PMA + MED with PMA + HIS, 10^{-4} M, yielded differences significant at $P < 0.05$.

studies with cultured neuroblastoma cells, cultured hamster smooth muscle, pig tracheal membranes, and guinea pig brain [9, 10, 23, 24]. The [^{3}H]mepyramine binding site on hamster smooth muscle cells has been localized to a 38,000 molecular weight protein by Mitsuhashi and Payan [25]. Ruat and co-workers [24] utilized an ^{125}I-labeled photoaffinity probe derived from mepyramine to label a 56,000 molecular weight peptide from guinea pig brain which contains the ligand binding domain of the histamine H_1 receptor. The demonstration of single-site binding for mepyramine at physiologically relevant concentrations is important in order to eliminate the possibility of labeling non-receptor binding sites.

The observed [^{3}H]mepyramine K_D values of 7.6 and 9.6 nM obtained in saturation studies with PMA-treated and control cells, respectively, were in close agreement with the non-radioactive mepyramine K_D of 11.3 nM obtained in competition studies with PMA-treated cells. The K_D values for mepyramine obtained in this study are in reasonably close agreement with the results of Cameron and co-workers, who obtained a [^{3}H]mepyramine K_D of 3.8 nM using a whole cell binding assay with human peripheral blood monocytes [5].

Analysis of histamine competition curves with the LIGAND program yielded results consistent with two-site binding. The mean slope factor of 0.67 for these experiments was also consistent with multi-site binding. The dissociation constants of the high- and low-affinity states were 6.8 and 240 μM respectively. The high-affinity binding state was abolished in the presence of 100 μM GTPγS, thus suggesting the participation of a guanine nucleotide regulatory protein. Nakahata and co-workers [10] also found a similar effect of GTPγS on histamine binding in cultured neuroblastoma cells. Although these investigators did not analyze their data for multi-site binding, GTPγS treatment resulted in a rightward shift and a steeper slope of the competition curve for histamine, as was observed in our experiments. Similar observations on the effects of guanine nucleotides were reported in a study of histamine receptors of bovine aortic membranes [26]. Multi-site binding for histamine was also found in a previous study using pig tracheal membranes [9]. Using solubilized receptors from human mononuclear cells, Warlow and co-workers [2, 27] found three distinct affinity states for histamine binding with K_D values in the 10^{-9}, 10^{-7}, and 10^{-4} M range. Single-site binding for 2-methylhistamine, a selective H_1 agonist, was suggested by a slope factor of 0.92. The rightward shift of the concentration curve which reflects the lower potency of this agent relative to histamine may be responsible for this unexpected finding.

The range of 6,000–12,000 receptors per cell obtained in this study is similar to results obtained by Cameron and co-workers, who found an average of 5,589 receptors per cell in a study of human peripheral blood monocytes [5]. In that study, various subsets of peripheral blood lymphocytes were found to possess 6,800–36,000 receptors per cell. T-lymphocytes stimulated with concanavalin A or phytohemagglutinin increased their histamine H_1 receptors 4-fold over a 24-hr period. In another study of human mononuclear cells, Casale and co-workers [4] found two distinct classes of H_1 receptors, one having a [^{3}H]mepyramine K_D in the nanomolar range with a receptor number of 2,100 receptors per cell.

Treatment of U937 cells with PMA resulted in a doubling of cell receptor numbers to 12,000 receptors per cell along with the characteristic feature of a more differentiated phenotype, i.e. increased cell adherence to plastic surfaces. The increase in receptor number along with increased cell adherence to plastic was inhibited completely by the addition of actinomycin D or cycloheximide. These effects were dose dependent. Actinomycin D inhibits DNA-dependent RNA polymerase and cycloheximide binds to the 80s ribosomal subunit, thus preventing peptide bond formation. These results imply that the increase in receptor density is a result of *de novo* receptor synthesis in PMA-treated cells. The increase in histamine receptor density was not observed after only 4 hr of PMA treatment. Treatment with PMA for 48 hr resulted in an increased cell mortality (greater than 15%). The expression of histamine H_1 receptors on PMA-treated U937 cells thus paralleled the appearance of Fc receptors and macrophage-specific surface antigens [12]. These data suggest that PMA may increase histamine receptor number indirectly by stimulating the cells to differentiate into a more mature, monocyte-like cell. Alternatively, PMA may exert its effects on histamine receptors independent of its ability to stimulate differentiation. Further studies are required to

determine the mechanism by which PMA exerts these effects.

Incubation of native and PMA-differentiated cells with histamine for 24 hr resulted in a dose-dependent down-regulation of histamine H_1 receptors. Concentrations of histamine greater than 10^{-4} M resulted in an increase in cell mortality. Agonist-induced receptor down-regulation is a characteristic feature of other receptors, most notably β-adrenergic, epidermal growth factor, and insulin receptors [28–30]. A previous attempt to down-regulate T-lymphocyte histamine receptors resulted in reduced binding affinity for [^{3}H]mepyramine without change in receptor number per cell [5]. Incubation with histamine was only carried out for 30 min in this study.

One of the limitations of this study is that histamine competition curves were performed only with PMA-treated cells. Unfortunately, the lower number of receptors present in native and histamine-treated cells and the necessity of using a tritiated radioligand did not permit an accurate detailed assessment of binding states under these conditions. PMA has been reported to cause uncoupling (but not reduced receptor number) of α_1-adrenergic receptors in cultured hamster vas deferens smooth muscle cells [31]. Thus, it is possible that the results we observed in histamine receptors, which are biochemically very similar in action to α_1-adrenergic receptors, may have been affected by PMA.

While biochemical responses to receptor activation were not examined, these results suggest that a feedback mechanism causing reduced receptor expression occurs as a result of receptor occupation by histamine. This provides physiologic evidence that histamine receptors can be reliably identified with radioligand binding in these cells. Desensitization of the biochemical response to histamine has been described recently by Nakahata and Harden [32]. This desensitization response occurred within 5 min after receptor activation. It is of interest that both histamine and phorbol esters directly or indirectly activate protein kinase C [32, 33], yet diverse changes in receptor expression occur when U937 cells are exposed to these substances. It is possible that receptor down-regulation is mediated by guanine nucleotide binding proteins or products of phosphatidyl inositol hydrolysis other than diacylglycerol.

Reappearance of plasma membrane receptors was demonstrated in desensitized cells when histamine was removed from the medium and cycloheximide added. This observation suggests that down-regulation receptors are internalized and then recycled back to the plasma membrane when histamine is removed from the medium. No demonstrable [^{3}H]mepyramine binding was detected in the supernatant fraction of the crude membrane preparation derived from desensitized cells. This observation is in contrast with prior studies of down-regulated β-adrenergic receptors which can be recovered from internalized cytoplasmic vesicles [28]. The recent identification of functional H_2 receptors in U937 cells by Gespach and coworkers [34] raises the interesting possibility that this cell line may provide a model for the study of the interaction of H_1 and H_2 receptors. Cell differentiation was accompanied by a reduced capacity to generate cAMP upon stimulation with histamine, isoproterenol, and prostaglandin E_1. This suggests that H_1 and H_2 receptor responses are inversely related in native and differentiated U937 cells.

The results indicate that U937 cells may provide a unique model for the study of human histamine receptor regulation and gene expression. The presence of histamine H_1 receptors in this promonocytic cell line is consistent with previous observations of histamine receptor binding and function in mononuclear cells. The high specific binding, the highly reproducible nature of the assay, and the ease of maintaining the cells in continuous culture all help to overcome previous difficulties in the study of histamine H_1 receptors. Further work is needed to determine the mechanism of PMA effects on histamine H_1 receptors and to determine the identity and function of guanine nucleotide binding proteins in this receptor system.

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