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PHARMACOLOGICAL CHARACTERIZATION OF HISTAMINE H_2 RECEPTORS ON CLONAL CYTOLYTIC T LYMPHOCYTES

EVIDENCE FOR HISTAMINE-INDUCED DESENSITIZATION*

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Abstract—Cultured cytolytic T lymphocytes of clonal origin were screened for histamine-stimulated cyclic AMP production. Histamine caused a 2- to 8-fold elevation of cyclic AMP levels in five independent clones. The EC_{50} for histamine of 1.7×10^{-5} M and the rank order of potencies of H_1 and H_2 agonists [impromidine > histamine > dimaprit > 4-methylhistamine > 2-methylhistamine > 2-(2-aminoethyl)-thiazole] were characteristic of the conventional histamine H_2 receptor. H_1 and H_2 antagonists inhibited histamine-stimulated cyclic AMP elevation with inhibition constants typical for those found on other H_2 receptor systems. Prior incubation of cells with histamine resulted in a marked loss in responsiveness to subsequent histamine challenge. We demonstrate that this desensitization is dose and time dependent and results in a change in the efficacy and not the potency of histamine. Although cyclic AMP increases could also be elicited with isoproterenol, prostaglandin E_1 or forskolin, desensitization of histamine had no effect on the ability of these agents to stimulate cyclic AMP production. In contrast to the rapid rate of histamine-induced desensitization, recovery of histamine responsiveness could not be detected for several hours.

Histamine H_2 receptors, coupled to adenylate cyclase, have been described in diverse cell types including brain [1], cardiac ventricular muscle [2], gastric mucosal cells [3], and peripheral blood lymphocytes [4]. Histamine interaction with the H_2 receptor of lymphocytes results in the inhibition of cellular inflammatory responses. Studies *in vitro* show that histamine inhibits cytotoxic T cell activity [5], activates suppressor T cell release of suppressor factors [6] and inhibits the production of macrophage inhibitory factor [7]. Although it has not been demonstrated conclusively that it is the histamine-elicited increase in cyclic AMP which mediates these effects of H_2 receptors, the demonstration that other agents which elevate cyclic AMP, such as prostaglandin E_1 (PGE_1‖), isoproterenol, or phosphodiesterase inhibitors [8], also inhibit many lymphocyte functions supports this hypothesis.

For a variety of hormones it has been demonstrated that desensitization, a loss of responsiveness, occurs upon prolonged exposure of receptive cells to the appropriate hormone. In fact, regulation of lymphocyte histamine sensitivity may occur as a consequence of persistent stimulation by histamine. Lymphocytes derived from atopic individuals who have demonstrably elevated circulating histamine [9] have a diminished responsiveness to histamine in cytotoxicity assays [10] as well as in the generation of suppressor cell activity [11]. Intraperitoneally administered histamine has also been shown to decrease the responsiveness of murine splenic T lymphocytes to subsequent histamine exposure [12]. The mechanism underlying these attenuations of histamine responsiveness has not been established but would be consistent with a desensitization phenomenon [13].

To date, examination of the immunological function of histamine has been conducted exclusively in heterogeneous populations of lymphocytes. Thus, despite extensive investigation, the distribution of H_2-receptor subtypes among lymphocyte subpopulations and, therefore, the cellular sites of histamine anti-inflammatory actions are unknown. This has hampered and complicated studies of histamine control of lymphocyte function. To resolve these issues, we examined the pharmacological properties of several non-transformed immunocompetent, cytolytic T cell clones. These clones are shown to have a histamine sensitive adenylate cyclase with pharmacological characteristics of H_2-receptors. The desensitization of the H_2-receptor observed in these cells provides a model of the regulation *in vivo* of lymphocyte sensitivity to histamine.

MATERIALS AND METHODS

Histamine dihydrochloride, isoproterenol, 3-isobutyl-1-methylxanthine (IBMX), cyclic AMP,

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‖ Abbreviations: PGE_1, prostaglandin E_1; IBMX, 3-isobutyl-1-methylxanthine; PBS, phosphate-buffered saline; TEA, 2-(2-aminoethyl)thiazole; HEPES, 4-(2-hydropyethyl)-1-piperazine-ethanesulfonic acid; and FCS, fetal calf serum.

PGE_1, and O^2-monosuccinyl-adenosine 3′:5′-cyclic monophosphate tyrosyl methyl ester were purchased from the Sigma Chemical Co. (St. Louis, MO). ^{125}I was purchased from the New England Nuclear Corp. (Boston, MA). Cimetidine, metiamide, mepyramine maleate, 4-methylhistamine dihydrochloride, 2-methylhistamine dihydrochloride, and 2-(2-aminoethyl)thiazole dihydrochloride (TEA) were donated by Dr. C. R. Ganellin (Smith Kline & French, Research Ltd., Welwyn Garden City, U.K.). Tiotidine was provided by Dr. T. O. Yellin (ICI Americas Inc., Wilmington, DE) and promethazine by Wyeth Laboratories, Inc. (Philadelphia, PA). Antibodies to cyclic AMP were obtained from Dr. G. Brooker (Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, VA). All other compounds were reagent grade.

Cell origins and method of cell culture. Cytolytic T cell clones 3A1, C4.2, and B12 were generated using previously described methods [14, 15]. C57BL/6 spleen cells were sensitized to BALB/c alloantigens using a one-way mixed lymphocyte culture. After six generations of restimulation, the responding cells were cloned by limiting dilution in the presence of irradiated BALB/c stimulator cells and rat concanavalin A supernatant fraction as a source of interleukin-2 (IL-2). After recloning, the cells were propagated by weekly restimulation of 4×10^5 responding cells (clone) with 2.5×10^7 irradiated (3100 rads) BALB/c spleen cells in 5 ml of medium in upright 25 cm^2 flasks. The medium consisted of RPMI 1640 with 5×10^{-5} M 2-mercaptoethanol, glutamine, 10% bovine serum (Zeta serum -D), and 10–12% concanavalin A supernatant fraction. These clones are highly cytotoxic for target cells bearing H-2^d alloantigens.

Clones AR-1 and 1E4, provided by Dr. E. Pillemer, Stanford University, are from C57L mice primed *in vivo* with the Abelson virus-induced tumor cell lines RAW 112 and L1-2 respectively. Spleen cells were restimulated *in vitro*, cloned, and propagated using methods similar to those just described. AR-1 is specifically cytotoxic for H-2^d target cells while 1E4 recognizes a cell surface Abelson virus determinant in the context of H-2^b antigens.*

Stimulation of cells and assay of cyclic AMP levels. Cells were harvested by centrifugation at 800 *g* for 3 min, washed once, and resuspended in phosphate-buffered saline (pH 7.5, 37°) containing 0.5 mM IBMX. In a standard assay, cell suspensions ($2–4 \times 10^5$ cells/ml) were preincubated at 37° for 10 min with continuous gentle agitation. The reaction was initiated by the addition of 225 μl of the cell suspension to 25 μl of drug in 12 × 75 mm glass tubes. The incubation was terminated after 1 min by the addition of 25 μl of 1 N HCl. Assay tubes, after storage at −20° for periods up to 1 week, were thawed, and samples were neutralized with 25 μl of 1 N NaOH. To acetylate cyclic AMP, a 10-μl aliquot of a triethylamine–acetic anhydride (2:1) mixture was added to each sample, and the tube was rapidly vortexed. The cyclic AMP content of 100-μl aliquots was determined by the radioimmunoassay procedure of Brooker *et al.* [16] using [^{125}I]tyrosylsuccinyl-cyclic AMP prepared by the cholarimine T method as described by Steiner *et al.* [17]. Standards of cyclic AMP were diluted in the incubation buffer and subjected to the same procedure of acidification, neutralization, and acetylation.

Desensitization and its reversal. Cells in culture media (RPMI-1640, 10% bovine serum containing 25 mM HEPES, pH 7.5) were pretreated with various concentrations of histamine for appropriate time intervals at 37°. Desensitization was terminated by quickly diluting cells with 10 vol. of PBS. Histamine was removed by two washes of the centrifuged cells (5 min, 800 *g*) using the same buffer. Cells were resuspended in PBS containing 500 μM IBMX at 37°, and the residual ability of histamine to stimulate cyclic AMP accumulation was assayed by adding cells to histamine for 1 min, stopping the reaction with 1 N HCl, and measuring cyclic AMP as described above. Cell suspensions were recounted after the final resuspension to correct for cell loss through the centrifugation steps.

To examine the reversal of desensitization, cells were desensitized as described above, washed twice, resuspended in RPMI-1640, 10% FCS with 25 mM HEPES (pH 7.5), and maintained at 37° with 5% CO_2 atmosphere. At various times thereafter, aliquots were removed, diluted in PBS containing 500 μM IBMX, and restimulated with 1 mM histamine. Cyclic AMP levels were determined as described above.

RESULTS

Screening of clones. Five independent cytolytic T cell clones and two lymphomas were screened for histamine-stimulated cyclic AMP production. As shown in Table 1, all cells had basal cyclic AMP levels of 1–2 pmoles/10^6 cells in the presence of 200 μM IBMX. Addition of 50 μM histamine had no significant effect on the cyclic AMP levels of the two lymphomas. In contrast, all five of the cytolytic T cells (C4.2, B12, 1E4, 3A1, and AR1) responded with a 2- to 3-fold increase in cyclic AMP (Table 1). Each of the five clones had linear log dose–response curves with half-maximal responses, EC_{50} values in the range $1–3 \times 10^{-5}$ M. This effect of histamine was consistently seen in all five cell lines, although variations in maximal fold stimulation did occur between experiments. Variability in the stimulation of adenylate cyclase has been reported previously in other cell lines [18]. Clones C4.2 and 1E4 were chosen for further study. These cell lines were used interchangeably as no differences in drug sensitivities between C4.2 and 1E4 cells were ever noted.

Effects of various H_1 and H_2 receptor agonists and antagonists on cyclic AMP production. The effects of histamine and H_1 and H_2 receptor agonists and antagonists were studied in order to characterize the cyclic AMP response. In the presence of 200 μM IBMX, histamine increased cyclic AMP production in a dose-dependent manner (Fig. 1). This stimulation was observed at a concentration as low as 5×10^{-7} M and reached maximal levels at 0.5 to 1.0×10^{-3} M. The EC_{50} was $1.7 \pm 0.7 \times 10^{-5}$ M (N = 12). The H_2 agonists impromidine, 4-methylhistamine and dimaprit and the H_1 agonists 2-

* E. Pillemer, manuscript in preparation.

Table 1. Effect of histamine on cyclic AMP levels in clonal cytolytic T lymphocytes and lymphoma cell lines*

	Cyclic AMP (pmoles/10^6 cells)	
	Basal	50 μM Histamine
Cytolytic T cell clones		
C4.2	1.7	3.5
B12	1.2	4.1
AR1	1.9	4.9
1E4	1.4	5.1
3A1	1.2	3.8
Lymphoma cell lines		
EL4	1.5	1.5
L691	1.5	2.1

* Cells were harvested, washed, and resuspended in PBS containing 200 μM IBMX at 1×10^6 cells/ml. Aliquots were treated with 50 μM histamine for 1 min and subsequently analyzed for cyclic AMP content by radioimmumoassay.

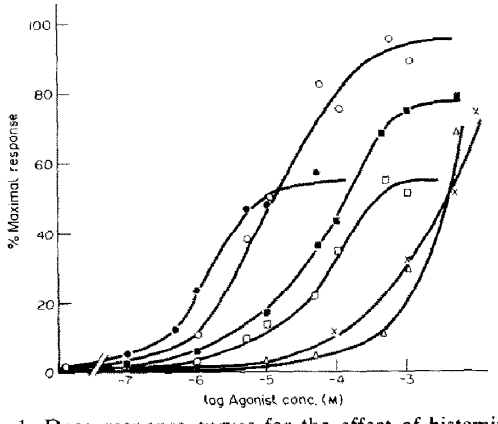

Fig. 1. Dose–response curves for the effect of histamine and H_1 or H_2 agonists on cyclic AMP production in cytolytic T cell clones. Cell were incubated in PBS containing 200 μM IBMX and treated with the indicated concentrations of histamine (○), impromidine (●), dimaprit (■), 4-MG (□), 2-MX (×), and TEA (△). Cyclic AMP levels were determined as described in Materials and Methods. Data are means from twelve experiments for histamine and three to four experiments for the H_1 and H_2 agonists. Each assay was performed in triplicate.

methylhistamine and TEA also stimulated cyclic AMP production in a dose-dependent manner. The EC_{50} values for these agonists were 1.42×10^{-6} M, 3.8×10^{-5} M, 7.7×10^{-5} M, 2.8×10^{-3} M, and $> 10^{-3}$ M respectively. The relative potency of each agonist can be defined as a ratio (EC_{50}) for histamine/EC_{50} for the agonist) by assigning a value of 100 for histamine. In Table 2, the relative potencies of the analogues impromidine (1200): histamine (100): dimaprit (44): 4-methylhistamine (23): 2-methylhistamine (0.67): TEA (<1) are compared to values obtained in other H_1 and H_2 receptor systems.

Impromidine, 4-methylhistamine, and dimaprit had EC_{50} values consistent with activation of H_2-receptors but appeared to be partial agonists (Fig. 1). Maximally effective concentrations of these drugs elicited responses that were 52, 58 and 71% of the histamine response (N = 3) respectively.

The inhibition of histamine-induced cyclic AMP formation by various H_1 and H_2 antagonists was studied by adding a fixed concentration of antagonist to different concentrations of histamine. The dose–response curves for histamine exhibited parallel shifts to the right following treatment with the H_2

Table 2. Comparison of the relative potencies of histamine H_1 and H_2 receptor agonists in cytolytic T lymphocytes and in other systems*

	Relative potencies			
Agonist	Cyclic AMP levels in cytolytic T lymphocytes	H_2 function gastric secretion in rat†	H_2 function cyclic AMP levels in human polymorphonuclear neutrophils‡	H_1 function Ileum contraction in guinea pig†
Histamine	100	100	100	100
Impromidine	1200	1680	1500	<0.001
Dimaprit	44	19.5		0.001
4-Methylhistamine	23	39	30	0.03
2-Methylhistamine	0.67	2		16.5
2-(2-Aminoethyl)thiazole	<1.0	0.3	1.4	26

* Relative potencies were calculated according to the equation: R.P. = (EC_{50} histamine/EC_{50} agonist) ×100.
† Ganellin [19].
‡ Gespach and Abita [20].

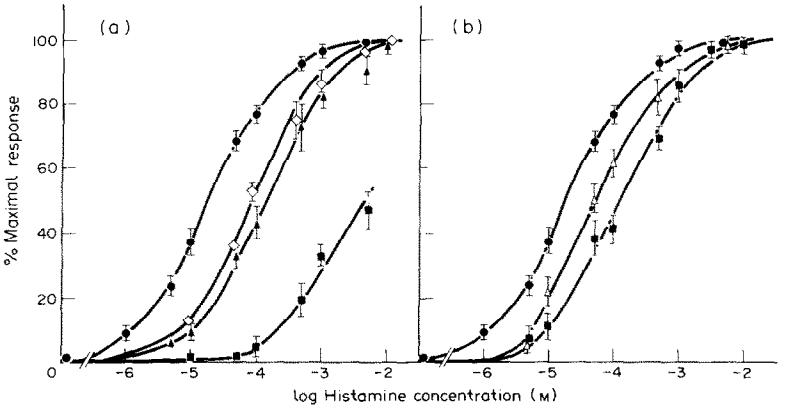

Fig. 2. Effect of histamine H_1 or H_2 receptor agonists on histamine-stimulated cellular cyclic AMP production in cytolytic T cell clones. Cells were incubated in PBS containing 200 μM IBMX with various concentrations of histamine alone (●) or with histamine plus the indicated antagonists. (A) 10^{-5} M cimetidine (▲), 10^{-5} M metiamide (◇), and 10^{-5} M tiotidine (■). (B) 5×10^{-5} M mepyramine (■) and 1×10^{-5} M promethazine (△). Data are the means ± standard error of the mean from N = 12 experiments for histamine and N = 3 experiments for the H_1 and H_2 antagonists.

antagonists cimetidine, metiamide, and tiotidine (Fig. 2A), and with the H_1 antagonists mepyramine and promethazine (Fig. 2B). The inhibition constants calculated for each of these drugs are shown in Table 3 and compared to the inhibition constants found for these drugs in other conventional H_1- and H_2-receptor systems.

Histamine stimulation of T_{cyt} cells—Effect of time and IBMX concentration on cyclic AMP levels. In the absence of IBMX, the basal level of cyclic AMP in clone C4.2 was 0.41 pmole/10^6 cells. Addition of 1 mM histamine resulted in a time-dependent increase in cyclic AMP, reaching a maximal value of 2.0 pmoles/10^6 cells or a 5.3-fold stimulation with a 1- to 2-min incubation. The ability to elevate cyclic AMP levels declined gradually as the incubation time was increased, such that by 30–60 min the cyclic AMP levels attained were only 0.1 to 0.2 pmole/10^6 cells above basal (Fig. 3). Addition of IBMX at 200 and 500 μM raised the basal levels of cyclic AMP to 1.0 and 1.2 pmoles/10^6 cells respectively. Again, histamine caused a time-dependent increase in cyclic AMP, reaching a maximum at 2–3 min. Stimulations of 6.7- and 6.5-fold for 200 and 500 μM IBMX, respectively, were seen. Incubation with histamine for longer than 2–3 min resulted in levels of cyclic AMP which were less than maximal. This decline in cyclic AMP content during prolonged exposure to histamine, even in the presence of IBMX, is also seen in many other receptor-linked adenylate cyclase systems, where it appears to be a consequence of a decrease in the rate of cyclic AMP synthesis.

Effect of histamine pretreatment on subsequent histamine responsiveness. The time course of his-

Table 3. Comparison of H_1 and H_2 receptor antagonist inhibition constants in T cytolytic lymphocytes and in other H_1 and H_2 receptor systems*

	K_i (μM)			
Antagonist	Cytolytic T lymphocyte	Guinea pig gastric cells (H_2)†‡	Guinea pig cardiac ventricular muscle (H_2)§	Ileum contraction guinea pig (H_1)‖
Cimetidine	1.45	0.81	0.96	398.0
Metiamide	2.15	1.41	1.4	
Tiotidine	0.04	0.02		
Mepyramine	2.6		5.2	0.0004
Promethazine	1.6	10.0	0.076	0.0012

* Inhibition constants (k_i) were calculated according to the equation:

$$k_i = \frac{I}{(k'_a/k_a) - 1}$$

[21] where k_a and k'_a are the concentrations of histamine required to give half-maximal activation of adenylate cyclase in the absence and presence of antagonist, respectively, and I is the concentration of the antagonist.

† Batzri *et al.* [3].

‡ Batzri and Gardner [22].

§ Kanof and Greengard [23].

‖ Johnson *et al.* [2].

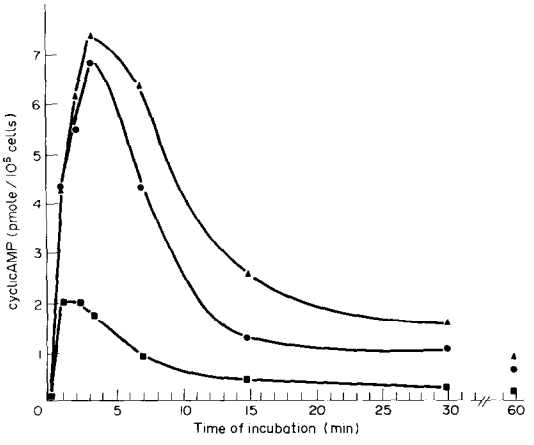

Fig. 3. Time course of cyclic AMP production induced by histamine using various concentrations of IBMX. Cells were treated with 1 mM histamine for various lengths of time. The amount of cyclic AMP produced was determined as described in Materials and Methods. IBMX concentrations were 0 μM (■), 200 μM (●), and 500 μM (▲). Each point represents the mean of triplicate determinations; this experiment is representative of two others.

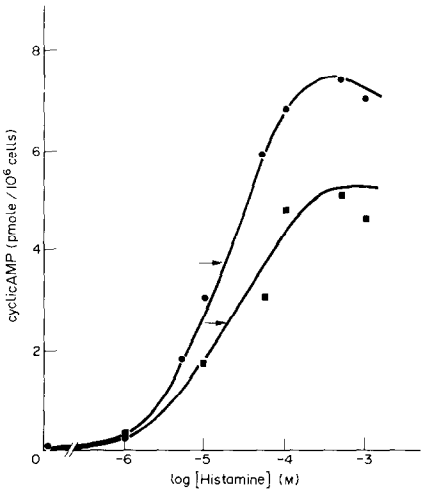

Fig. 4. Dose–response curve for histamine-stimulated adenylate cyclase activity in cytolytic T cells from control and desensitized cells. Cells were incubated for 10 min with 50 μM histamine (■), washed twice with fresh PBS, and incubated with PBS + IBMX for 10 min prior to histamine challenge. Control cells (●) were treated in a similar manner. Each point represents the mean of triplicate determinations; this experiment is representative of two similar experiments.

tamine-elicited cyclic AMP accumulation (Fig. 3) suggested that a desensitization of the H_2 receptor had occurred. To test this, C4.2 cells were preincubated in the absence or presence of histamine and then tested for histamine-stimulated cyclic AMP accumulation. In a typical experiment, after a 1-hr preincubation without histamine, challenge with 1 mM histamine for 1 min elevated cyclic AMP levels by 5–8 pmoles/10^6 cells (Table 4). In contrast, after a previous 1-hr preincubation with 1 mM histamine, C4.2 cells exhibited no response to the same challenge dose.

Desensitization appeared to alter the efficacy but not the potency of histamine. Cells were partially desensitized by a 10-min treatment with 50 μM histamine, washed to remove the desensitizing drug, and restimulated with appropriate doses of histamine. As can be seen from the dose–response relationships (Fig. 4), preincubation led to a (40%) decrease in maximal cyclic AMP accumulation, but had no significant effect on the EC_{50} for histamine.

The desensitization process for the histamine H_2 receptor was both dose and time dependent (Fig. 5). Cells were exposed to the indicated doses of histamine for varied lengths of time. After removal of the preincubation dose, the residual responsiveness of the H_2 receptor was examined by challenging cells with 1 mM histamine. Preincubation with high doses of histamine (500 μM) caused a rapid loss of responsiveness such that 50% of the control response ($T_{1/2}$) was observed after only a 5-min preincubation. Lower doses of histamine (5 μM) in the preincubation period caused a loss of responsiveness with a slower time course ($T_{1/2}$ = 27 min).

The dose of histamine required to induce a 50%

Table 4. Homotropic desensitization of histamine sensitive adenylate cyclase*

Preincubation condition	Challenge condition	Cyclic AMP (pmoles/10^6 cells)
PBS	PBS	0.4 ± 0.05
PBS	1 mM Histamine	6.2 ± 0.4
1 mM Histamine	1 mM Histamine	0.3 ± 0.8
PBS	0.1 mM Isoproterenol	4.4 ± 0.3
1 mM Histamine	0.1 mM Isoproterenol	4.0 ± 0.4
PBS	0.1 mM PGE_1	172.4 ± 15.6
1 mM Histamine	0.1 mM PGE_1	200.0 ± 25.8
PBS	0.01 mM Forskolin	7.9 ± 0.8
1 mM Histamine	0.01 mM Forskolin	9.5 ± 0.5

* C4.2 cells were incubated in the presence or absence of 1 mM histamine for 60 min. After thorough washing to remove histamine, cells were challenged with various agents for 1 min, and the cyclic AMP content was determined by radioimmunoassay. Values represent the mean ± standard deviation of triplicate determinations.

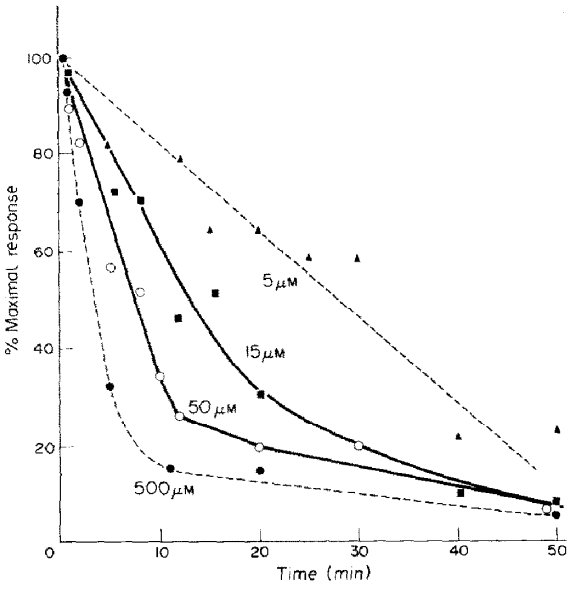

Fig. 5. Time course of desensitization of histamine-stimulated adenylate cyclase activity after short term incubation with various concentrations of histamine. Cells were incubated with 5 μM (▲), 15 μM (■), 50 μM (○), or 500 μM (●) histamine; at the indicated times cells were pelleted, thoroughly washed, and histamine-stimulated (1 mM) adenylate cyclase activity was determined. Responsiveness was calculated as the percentage of maximal response elicited in control cells which were incubated in the same manner in the absence of histamine.

desensitization was determined. In Fig. 6, the reciprocal of the $T_{1/2}$ values obtained from Fig. 5 was plotted as a function of the histamine dose. The concentration of histamine required to produce half-maximal desensitization (EC_{50}) was 2.5×10^{-5} M, similar to the EC_{50} for stimulation of adenylate cyclase (1.7×10^{-5} M).

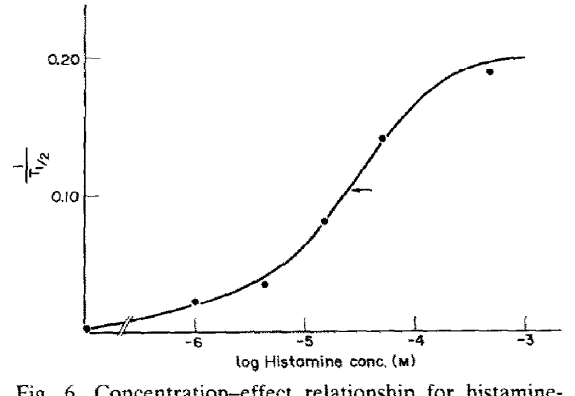

Fig. 6. Concentration–effect relationship for histamine-stimulated desensitization in cytolytic T cells. Cells were treated as described in the legend of Fig. 5. $T_{1/2}$ values for histamine-induced desensitization were calculated from linear regression analysis of the linear portion of the desensitization plots (Fig. 5). Correlation coefficients of 0.77, 0.74, 0.95, and 0.87 were obtained for the initial linear portion of the plots obtained (Fig. 5) from the 5, 15, 50 and 500 μM pretreatments respectively. The reciprocal of the $T_{1/2}$ values was plotted as a function of histamine concentration. The EC_{50} value was approximately 2.5×10^{-5} M.

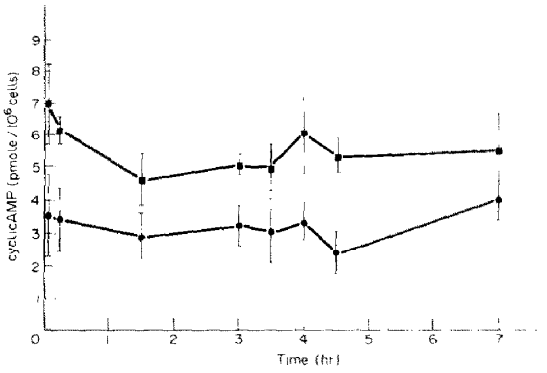

Fig. 7. Examination of histamine-stimulated adenylate cyclase responsiveness at time intervals subsequent to the initial histamine desensitization. Cytolytic T cells were treated with (●) or without (■) 500 μM histamine for 10 min; they were then thoroughly washed and resuspended in RPMI-1640 with 10% FCS and HEPES, pH 7.5. Cells were incubated at 37°, at 5% CO_2 atmosphere; at the indicated times, 1 mM histamine was added for 2 min and cyclic AMP levels were determined. The data are the mean ± standard deviation of triplicate determinations obtained from two experiments. Results are representative of five similar experiments using different desensitizing concentrations of histamine.

To assess the receptor specificity of the desensitization phenomenon, cells were exposed to histamine in the absence or presence of the H_2 antagonist cimetidine, washed, and then challenged with histamine. Cells preincubated with 100 μM histamine responded to a 1 mM histamine challenge with an increase of only 0.87 pmole cyclic AMP/10^6 cells, whereas cells preincubated with PBS or with 100 μM histamine plus 1 mM cimetidine responded to the challenge with an increase of 11.0 and 11.8 pmoles cyclic AMP/10^6 cells respectively. Cimetidine, at a 10-fold higher concentration than histamine, prevented the histamine-induced desensitization. Mepyramine at a concentration that completely blocks H_1 receptors (10^{-6} M) had no effect on desensitization. In addition, the desensitization elicited by histamine was homologous, i.e. no effects on isoproterenol, PGE_1, or forskolin stimulation of cyclic AMP were observed (Table 4).

We examined the ability of the cells to recover hormonal responsiveness after a previous desensitization. When cells were fully desensitized (90–100%), no recovery of responsiveness was noted. Even when C4.2 cells were only partially desensitized (40–50%), there was negligible recovery after 7 hr (Fig. 7). Subsequent experiments demonstrated no change from the desensitized state even after 24 hr (data not shown).

DISCUSSION

In previous studies, analysis of the interrelationship between cytolytic T cell function and hormonal responsiveness has been complicated by the loss of histamine sensitivity within hours after initial culturing of splenic T lymphocytes [24]. Additionally, the use of heterogeneous mixed lymphocyte cultures has precluded a precise definition of the cellular

site(s) of histamine action. The data reported here demonstrate that clonal populations of cytolytic T cells, initially selected for their immunologic function and maintained in culture for several years, retain histamine responsiveness.

Five immunocompetent T cell clones were tested and shown to be positive for histamine-stimulated increases in cyclic AMP (Table 1). In contrast, two lymphomas tested for activity had no histamine-sensitive response. The ability of histamine to increase cyclic AMP accumulation in each of the clones tested leads us to believe that histamine receptors are generally found on murine cytolytic T lymphocytes. This finding is in conflict with the hypothesis [25] that histamine receptors are located solely on the suppressor T cell subpopulation, and it implies that histamine acts directly on cytolytic T lymphocytes thereby inhibiting cellular functions. This has been confirmed by more recent data (unpublished studies).

The results of the present study support the conclusion that the cytolytic T cell histamine receptor possesses pharmacological properties similar to those of the conventional H_2 receptor as defined by physiological experiments on peripheral tissues [26]. The concentration of histamine required for half-maximal cyclic AMP accumulation (EC_{50}) was found to range from 1 to 4×10^{-5} M ($\bar{x} = 1.7 \pm 0.7 \times 10^{-5}$ M, N = 12). The relative potencies of a variety of compounds as activators of cyclic AMP accumulation in cytolytic T cells agree with their relative potencies as agonists of physiological responses mediated by other H_2 receptors, but not H_1 receptors (Table 2). Impromidine was 12-fold more potent than histamine, which was 2.4-fold more potent than dimaprit and 4.9-fold more potent than 4-methylhistamine. The potencies of the H_1 agonists 2-methylhistamine and TEA were low, as has been observed in other H_2 receptor systems.

Although the partial agonism which we have observed with 4-methylhistamine and impromidine is not a universal phenomenon, there are several other studies documenting partial rather than full agonist activity. In guinea pig gastric mucosal cells [3] and in HL-60 cells [27], impromidine has 42 and 15–20% of the efficacy of histamine respectively. It has also been shown that 4-methylhistamine is only 43% as efficacious as histamine in the guinea pig atria assay [21]. The reason for these compounds being full agonists in some systems and partial agonists in others is not known, though it may reflect the presence or absence of spare receptors.

Antagonist studies of this adenylate cyclase-linked receptor define this receptor as the histamine H_2 type. The inhibition constants for the H_2 receptor antagonists tiotidine, cimetidine, and metiamide were 0.04, 1.45 and 2.15 μM, respectively, for inhibition of histamine-stimulated cyclic AMP accumulation in cytolytic T cells. In Table 3 these numbers can be seen to be comparable to values found at H_2 receptors from guinea pig gastric mucosal cells [3, 22] and homogenates of cardiac ventricular muscle [23]. The H_1 antagonists, mepyramine and promethazine, had inhibition constants of 2.6 and 1.6 μM, in agreement with the values found at H_2 receptors in guinea pig ventricular muscle [23] and in brain slices [28]. Interestingly, the pharmacology of H_2 receptors measured in cell-free and intact cells differs. In particular, promethazine is approximately 200-fold more potent as an inhibitor of adenylate cyclase activity in rat brain homogenates than as an inhibitor of cyclic AMP accumulation in brain slices [28]. Our findings for promethazine, using intact cytolytic T cells, correspond to those seen with other intact systems [22, 28]. These values indicate potencies about 100-fold less than in cell-free systems such as homogenates of cardiac ventricular muscle [23] or brain [28]. While mepyramine and promethazine were relatively potent H_2 receptor antagonists, the observed inhibition constants are 1,000- to 10,000-fold higher than the inhibition constants of 0.5 nM found for inhibition of H_1 receptor-mediated contraction of guinea pig ileum [29], as can be seen in Table 3.

The elevation of cyclic AMP levels by histamine agonists was transient. The rapid rise in cyclic AMP content was followed by a gradual decline, with nucleotide levels returning to near basal concentrations within 30–60 min. This decay in cyclic AMP levels occurred even though the concentration of IBMX was increased from 0 to 500 μM. A similar decrease of cyclic AMP levels as a function of histamine incubation time was seen by Study and Greengard in sympathetic ganglia [30] and by Kakiuchi and Rall in rabbit brain [1]. This rapid loss of histamine responsiveness suggested that desensitization of the receptor had occurred.

The loss of histamine responsiveness was therefore examined by pretreating the cells with agonist, followed by washing and rechallenge. The second exposure resulted in a diminished level of cyclic AMP (Fig. 4). Further study of the properties of the histamine-induced desensitization showed that this phenomenon was characterized by a maximal 90–100% loss of histamine responsiveness (Fig. 5) with no change in the sensitivity to histamine as measured by the EC_{50} (Fig. 4). Loss of responsiveness to histamine has been documented previously for H_1 receptors. The ability of H_1 receptor stimulation to elevate cyclic GMP in neuroblastoma cells [31] and mouse cerebral cortex [32] is attenuated by exposure to histamine. The characteristics of H_2 receptor desensitization were similar to the desensitization observed with the β-adrenergic receptor of astrocytoma cells [33], and the H_1 receptor of neuroblastoma cells [31], in that (1) the event was dose and time dependent (Fig. 5), (2) the dose required for half-maximal desensitization (2.5×10^{-5} M) was approximately equal to the EC_{50} for cyclic nucleotide stimulation (Fig. 6), (3) antagonists specific for the receptor (e.g. cimetidine) prevented desensitization and (4) no effect on the accumulation of cyclic nucleotides by other hormones occurred (Table 4).

The rate of desensitization was dose dependent (Fig. 5). Low doses of histamine desensitized more slowly than high doses. The dose of histamine required for half-maximal desensitization was 2.5×10^{-5} M, a value similar to the EC_{50} for cyclic AMP accumulation which was 1.7×10^{-5} M. This correlation and the observation that cimetidine blocked histamine-induced desensitization imply that desensitization resulted from the normal acti-

vation of the H_2 receptor by histamine. Histamine desensitized the cytolytic T cell in a homologous manner, i.e. preincubation with histamine for 1 hr had no effect on the elevation of cyclic AMP induced by other hormones such as isoproterenol and PGE_1 or the drug forskolin (Table 4). To our knowledge this is the first cell culture system where histamine-induced desensitization of H_2 receptors has been described.

The rapidity of the desensitization is reminiscent of the time course of desensitization of β-adrenergic receptors, in which an uncoupling between the receptor and the nucleotide binding protein has been postulated [34, 35] to subserve the loss of responsiveness. However, unlike the β-adrenergic receptor of astrocytomas [34], recovery of histamine responsiveness after administration of a desensitizing dose of histamine was negligible. In Fig. 7 it is apparent that receptor responsiveness remained depressed over a time course of 7 hr. This prolonged refractoriness occurred also in cells desensitized by 20–90% (data not shown). This protracted desensitization of the H_2 receptor of T cytolytic cells may reflect adaptation to repeated or prolonged histamine exposure *in vivo*.

A great deal of evidence supports a role for histamine as a modulator of immunological function *in vitro*. Further studies of the H_2 receptor distribution on lymphocytes, as well as studies of the pharmacological properties of this adenylate cyclase coupled receptor, may help to elucidate some of the fundamental biochemical and physiological events underlying the anti-inflammatory action of histamine. These clonal, cytolytic T lymphocytes will facilitate studies relating hormonal action with effector function, as well as providing a cell culture model for analysis of the mechanism of desensitization.

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