MOLECULAR PHARMACOLOGY, 22:33–40

Interaction of Histamine with Gastric Mucosal Cells

Effect of Histamine Agonists on Binding and Biological Response

SHMUEL BATZRI, JOHN W. HARMON, AND WILLIAM F. THOMPSON

Departments of Pharmacology and Surgery, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20014

Received December 4, 1981; Accepted February 17, 1982

SUMMARY

In dispersed cells from guinea pig fundic mucosa, histamine and each of eight chemically related analogues inhibited binding of [^{3}H]histamine and caused a 2- to 18-fold increase in cellular cyclic AMP. The rank order of potencies of these agonists on both processes were as follows: impromidine > dimaprit > histamine > 4-methylhistamine > 2-methylhistamine > 2-thiazolylethylamine > 2-pyridylethylamine > telemethylhistamine. The relative efficacy (i.e., maximal response) on cyclic AMP generation were histamine = dimaprit > 4-methylhistamine > 2-methylhistamine > 2-thiazolylethylamine > 2-pyridylethylamine > impromidine > nordimaprit > telemethylhistamine. Although the potency of some agonists for cyclic AMP generation did not agree with that for inhibition of [^{3}H]histamine binding, at sufficiently high concentrations all agonists abolished binding of [^{3}H]histamine. These results confirmed earlier reports that the actions of these agonists reflect their interaction with histamine H_2 receptors to activate a common catalytic moiety of adenylate cyclase located on the parietal cells. Impromidine, a specific and highly selective H_2 agonist, was a partial agonist and its potency as an agonist was equal to its potency as an inhibitor of the action of histamine on cyclic AMP, suggesting that impromidine and histamine interact with the same class of receptors. We compared the ability of the agonists to inhibit [^{3}H]histamine binding and to increase cyclic AMP. We found that with histamine, 4-methylhistamine, 2-methylhistamine, 2-thiazolylethylamine, and 2-pyridylethylamine there was a linear relationship between their ability to inhibit [^{3}H]histamine binding and their ability to stimulate cyclic AMP synthesis, and the slope of the line describing this relationship was approximately 1. In contrast, with impromidine, dimaprit, and nordimaprit the line describing this relationship was nonlinear. Higher concentrations of impromidine or dimaprit were required for half-maximal inhibition of binding than for half-maximal stimulation of cyclic AMP, whereas the inverse was the case with nordimaprit. Performing a direct comparison by plotting the log (potency) for inhibition of [^{3}H]histamine binding against the log (potency) for cyclic AMP stimulation resulted in two significantly different regression lines. We concluded that these two lines reflect the presence of two distinct classes of binding sites for [^{3}H]histamine that may have different affinities for the various agonists. Occupation of one site (i.e., the H_2 receptor) is sufficient to stimulate the generation of cyclic AMP.

INTRODUCTION

Histamine and chemically related analogues have been shown to stimulate acid formation and synthesis of cyclic AMP in gastric mucosal preparations of several species (1–5) and to increase cyclic AMP and [^{14}C]aminopyrine uptake in isolated gastric cells (6–13). These agonists interact with specific receptors to produce their biological effects. Detailed investigations of the structural requirements of gastric receptors have been performed on a variety of biological preparations ranging from intact animals to isolated cells (1, 3–6, 14–16).

We have previously reported that [^{3}H]histamine binds specifically to H_2 receptors on guinea pig gastric cells and that its binding correlated with the increase in cellular cyclic AMP and [^{14}C]aminopyrine uptake (7, 17). We found neither evidence for more than one class of receptors nor the existence of cooperative interaction between histamine and the receptors. However, a single straight line in a Scatchard plot (bound/free versus bound) (18) could be obtained in a system where more than one class of receptors coexist (19). Furthermore, the biological response as represented by measurement of cyclic AMP or acid formation may not necessarily reflect the events on the membrane receptors. Therefore, the use of hormone-responsive cell preparations offers the advantage

0026-895X/82/040033-08$02.00/0

of allowing measurements of biological response under conditions wherein direct binding to the receptors could be determined.

In the present studies we characterized the histamine receptors on mucosal cells isolated from guinea pig stomach by measuring the effects of several histamine receptor agonists on binding of [^{3}H]histamine and on stimulation of cellular cyclic AMP. Our results are compatible with the presence of two distinct binding sites for histamine on guinea pig gastric cells.

EXPERIMENTAL PROCEDURES

Materials

Male Hartley albino guinea pigs (200–250 g) were obtained from Camm Research Center (Wayne, N. J.). [^{3}H]Histamine (7.5–10 Ci/mmole), ^{125}I-labeled succinyl cyclic AMP tyrosine methyl ester (150 Ci/mmole), cyclic AMP antiserum (prepared with a second antibody), Triton X-100, and liquid scintillation fluid (Aquasol) were obtained from New England Nuclear Corporation (Boston, Mass.); histamine and theophylline were obtained from Sigma Chemical Company (St. Louis, Mo.); cimetidine, IMP,[1] 4-MH, 2-MH, PEA, TEA, TMH, DPT, and NDPT were gifts from Dr. C. R. Ganellin, Smith Kline & French Laboratories (Welwyn Garden City, Herts., England). Hanks' buffer was obtained from GIBCO (Grand Island Biological Company, Grand Island, N. Y.) or was prepared in our laboratory. The standard solution was a modification of Hanks' buffer and contained 137 mM NaCl, 5.37 mM KCl, 1.26 mM $CaCl_2$, 0.47 mM $MgCl_2$, 0.41 mM $MgSO_4$, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 5.5 mM glucose, 2.0 mM glutamine, 0.001% (w/v) phenol red, BME vitamin solution (GIBCO), 15 mM $NaHCO_3$, and 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4).

Methods

Dispersed gastric cells from guinea pig stomach were prepared as described previously (20). Unless otherwise indicated, the dispersed cells were collected by centrifugation, washed twice, and resuspended in standard solution. The biochemical and morphological characteristics of the cells were as previously described (7).

Binding of [^{3}H]histamine. Binding of [^{3}H]histamine to gastric cells was determined by our centrifugation technique as previously described (8, 17). In short, gastric cells (2–4 × 10^6 cells) were incubated in 0.5 ml of standard solution containing 0.1–0.25 μCi of [^{3}H]histamine for 40 min at 37°. The cells were washed twice by resuspension in 5 ml of iced standard solution followed by centrifugation (250 × *g* for 1 min) and resuspension. The washed cells were suspended in 1 ml of 1% (v/v) Triton X-100 and then dispersed in Aquasol. To determine specific binding of [^{3}H]histamine, 1 mM histamine was added to parallel incubations, and cell-associated radioactivity in these incubations was subtracted from the total observed binding to obtain specific binding. Typically, cells would be suspended in 20–40 ml of standard solution. Incubation of 0.5 ml of cell suspension with about 100,000 cpm of [^{3}H]histamine alone would result in cell-associated radioactivity of 1.3–3.8% of the total counts depending on the cell concentration. Excess of unlabeled histamine would reduce the cell-associated radioactivity by 80–85% (i.e., 250–650 cpm). In each experiment, incubations were carried out in triplicate, and the variation among these replicates was 10% or less. The variations between experiments could be as much as 50% in terms of absolute counts, but since we did not count the cells in each experiment, these variations reflect mostly the variations in cell number. These variations were reduced to 20% or less when corrected for cell number. Results are expressed as percentage of the saturable portion of [^{3}H]histamine at the beginning of the incubation.

Cellular cyclic AMP. Cyclic AMP was determined by radioimmunoassay as previously described (21). Cells were suspended in standard solution containing 5 mM theophylline, which did not alter the potency with which histamine or its analogues increase cyclic AMP (7). Since cyclic AMP became constant after a 15- to 20-min incubation, all incubation media contained 0.5 ml of cell suspension plus the indicated agents; incubation were carried out for 30 min at 37°.

Mathematical analysis of results. Results on cyclic AMP obtained with each agonist tested were compatible with agonist interaction with a single class of receptors. The values of $S_{0.5}$—the concentration of agonist required to produce a half-maximal response—and the values of R_{max}—the increase in cyclic AMP caused by the maximally effective concentration of agonist—were computed as described in detail previously (7). Although with a given agonist the values for R_{max} differed significantly from one experiment to another, the values for $S_{0.5}$ and the pattern of response with respect to other agonists did not. The values of *Kd*—the concentration of agonist required to produce a half-maximal inhibition of tracer binding—were calculated from experimentally determined values, using various transformations of the equation $F = H/(H + A + Kd)$, where H is the concentration of the tracer, A is the concentration of agonist, and F is the observed inhibition of tracer binding expressed as the fraction of the specific tracer binding obtained without the agonist (22).

RESULTS

Effect of histamine-receptor agonists on cyclic AMP. The chemical structure of the agonists we used and their relationship to histamine are illustrated in Fig. 1. In dispersed mucosal cells from guinea pig stomach, each of these agonists could cause up to a 20-fold increase in cellular cyclic AMP (Fig. 2). The most potent agonist was the guanidine derivative, IMP, which caused a detectable increase in cellular cyclic AMP at 0.02 μM. The least potent was TMH, which at 5 mM did not cause any significant increase in cellular cyclic AMP. If the potency[2] of histamine is assigned the value of 100, the relative

[1] The abbreviations used are: IMP, impromidine; 4-MH, 4-methylhistamine; 2-MH, 2-methylhistamine; PEA, 2-pyridylethylamine; TEA, 2-thiazolylethylamine; TMH, telemethylhistamine; DPT, dimaprit; NDPT, nordimaprit.

[2] "Potency" is measured in terms of the concentration of agonist required to produce a half-maximal response: the lower this concentration the higher the potency. $S_{0.5}$ and K_d are the potencies of an agonist on stimulating cyclic AMP generation and inhibiting tracer binding, respectively.

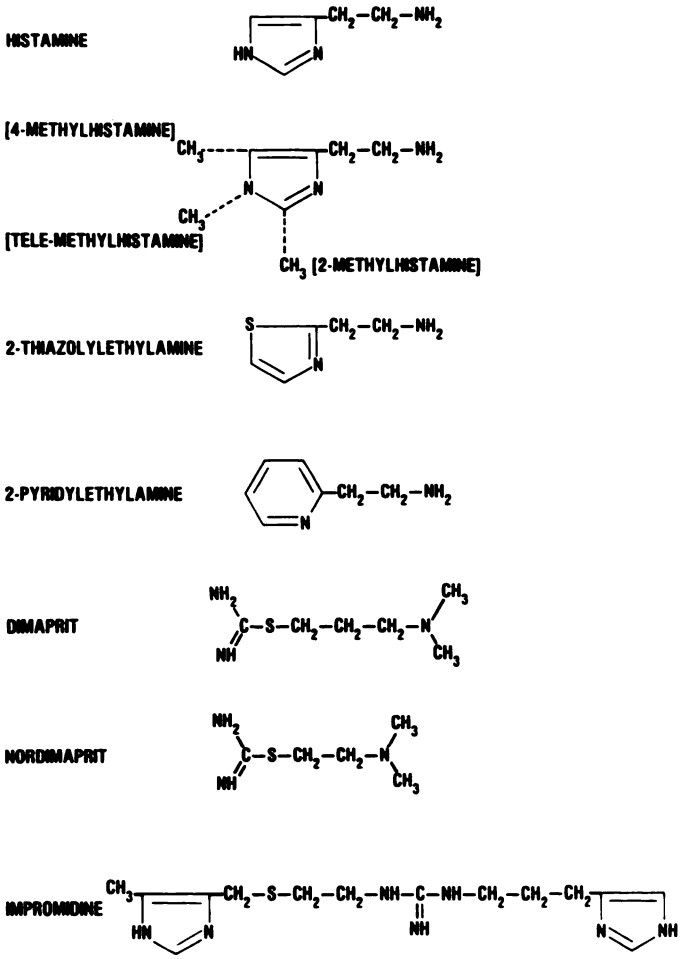

FIG. 1. *Chemical structures of histamine-receptor agonists*

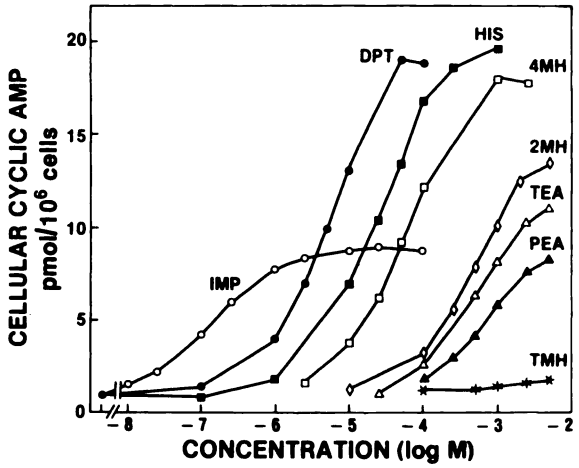

FIG. 2. *Effect of histamine-receptor agonists on cyclic AMP in dispersed mucosal cells from guinea pig stomach*

Cells were suspended in standard solution containing 5 mM theophylline and incubated with the indicated agents. Each point represents the mean of triplicate determinations; this experiment is representative of three others.

potencies of the agonists were as follows: IMP, 1570; DPT, 400; 4-MH, 60; 2-MH, 5; TEA, 4; PEA, 3; NDPT, 1; and TMH, <1. At maximally effective concentrations, histamine, 4-MH, and DPT each caused a 10- to 20-fold increase in cellular cyclic AMP. If the increase in cyclic AMP caused by the maximally effective concentration of histamine is assigned the value of 100, the maximal increases that could be obtained by the various agonists were as follows: DPT, 100; 4-MH, 100; 2-MH, 80; TEA, 70; PEA, 50; IMP, 40; and NDPT, 5 (Fig. 2; Table 1). To examine the response of two agonists in combination, we measured cyclic AMP in cells incubated with maximally effective concentrations of histamine alone and in combination with other agonists. We found that the increase in cyclic AMP caused by histamine plus any other agonist was equal to the increase caused by histamine alone.

IMP had about one-half the efficacy[3] of histamine in stimulating cyclic AMP generation (Fig. 2). If IMP is a partial agonist and interacts with the same receptors as histamine, it should behave as a competitive antagonist of the action of histamine on cyclic AMP. When these two agonists were tested in combination, IMP inhibited the action of histamine (Fig. 3). In the absence of IMP, an increase in cellular cyclic AMP could be detected with 2 μM histamine and was half-maximal at 15 μM histamine (Figs. 2 and 3A). In the presence of 1 μM IMP (which caused a 7-fold increase in cyclic AMP), 50 μM histamine was required to produce a detectable effect and 90 μM histamine was required to produce a half-maximal effect (Fig. 3). The value of $S_{0.5}$ for IMP, calculated from results obtained with IMP alone (Table 1; Fig. 2), was 0.14 ± 0.03 μM (mean ± standard deviation from three experiments). This value was not significantly different from

TABLE 1

Comparison of $S_{0.5}$ and R_{max} obtained by cyclic AMP analysis with K_d obtained by binding analysis

Values for cellular cyclic AMP were taken from Fig. 1 and ref. 6. All values are means ± standard deviation of number of experiments given in parentheses.

Agonist	Cellular Cyclic AMP		Binding, K_d[c]
	R_{max}[a]	$S_{0.5}$[b]	
		M	M
Histamine	100	$2.2 \pm 0.9 \times 10^{-5}$ (10)	$1.2 \pm 0.7 \times 10^{-5}$ (10)
4-MH	95 ± 9 (9)	$6.0 \pm 1.5 \times 10^{-5}$ (9)	$3.9 \pm 2.0 \times 10^{-5}$ (4)
2-MH	77 ± 6 (6)	$5.0 \pm 1.5 \times 10^{-4}$ (6)	$4.0 \pm 1.8 \times 10^{-4}$ (3)
TMH	5 ± 5 (3)	$>1.0 \times 10^{-3}$ (3)	$>2.0 \times 10^{-3}$ (3)
PEA	50 ± 8 (9)	$9.0 \pm 3.0 \times 10^{-4}$ (6)	$6.8 \pm 2.2 \times 10^{-4}$ (3)
TEA	73 ± 8 (4)	$7.0 \pm 2.0 \times 10^{-4}$ (4)	$7.1 \pm 2.0 \times 10^{-4}$ (3)
DPT	94 ± 6 (3)	$5.0 \pm 1.0 \times 10^{-6}$ (3)	$1.8 \pm 0.3 \times 10^{-5}$ (4)
NDPT	9 ± 4 (4)	$1.3 \pm 0.6 \times 10^{-3}$ (4)	$2.7 \pm 0.8 \times 10^{-5}$ (4)
IMP	42 ± 8 (3)	$1.4 \pm 0.3 \times 10^{-7}$ (3)	$4.7 \pm 2.1 \times 10^{-6}$ (3)

[a] The increase in cellular cyclic AMP caused by the maximally effective concentration of agonist, expressed as a percentage of the value for histamine.

[b] The concentration of agonist required to cause a half-maximal increase in cellular cyclic AMP.

[c] The concentration of agonist required to cause half-maximal inhibition of [^{3}H]histamine binding.

[3] "Efficacy" is measured in terms of the increase in cellular cyclic AMP caused by the maximally effective concentration of agonist and is defined as the sum of the intrinsic activities of all effector units when all binding sites are occupied (see ref. 23).

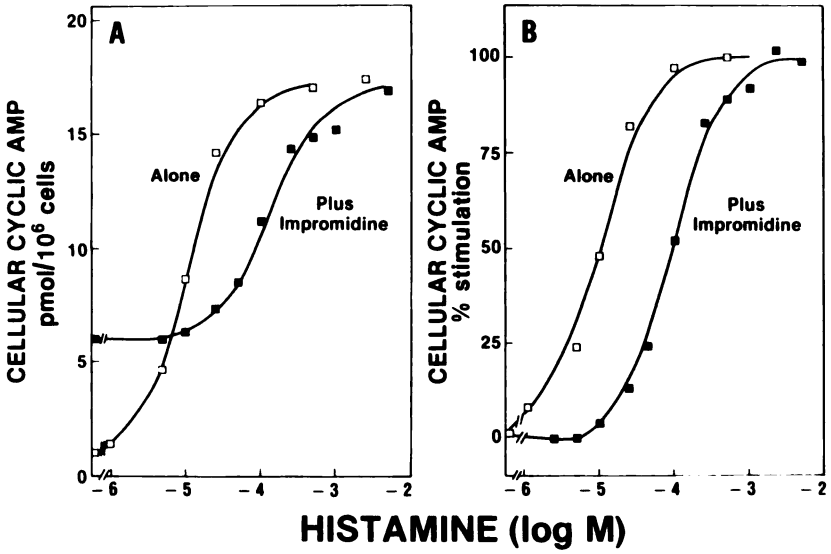

FIG. 3. *Effect of histamine and IMP on cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach*
A, Cells were incubated with or without 1 μM IMP plus the indicated concentration of histamine; B, cellular cyclic AMP is expressed as percentage of the maximal increase caused by histamine. Each point represents the mean of triplicate determinations; this experiment is representative of three others.

the value of K_i for IMP calculated from results obtained when IMP acted as a competitive antagonist of the action of histamine (i.e., 0.21 ± 0.6 μM) (Fig. 3). Similar results were obtained when DPT was used instead of histamine. IMP acted as a competitive antagonist to the action of DPT on cyclic AMP (results not shown).

Effect of agonists on [^{3}H]histamine binding. To explore the interaction between these agonists and the receptors mediating the increase in cyclic AMP, we examined their ability to inhibit binding of [^{3}H]histamine to dispersed mucosal cells. Each of the agonists inhibited binding of [^{3}H]histamine, and their rank order of potency (except for NDPT) was similar to the rank order of potency with which they increased cyclic AMP (Fig. 4). For example, in inhibiting [^{3}H]histamine binding, IMP (K_d 4.7 μM) was more potent than histamine (K_d 12 μM), which was more potent than 4-MH (K_d 39 μM). At sufficiently high concentrations, each agonist (except TMH) abolished binding of [^{3}H]histamine.

Although the rank orders of potencies of these agonists were similar for both processes, their absolute potency values were not. The potencies of IMP, DPT, and NDPT for stimulating cyclic AMP generation ($S_{0.5}$) were significantly different from their potencies for inhibiting [^{3}H] histamine binding (K_d). These differences were most notable with NDPT, a chemical analogue of the H_2 agonist DPT (Fig. 1) (14, 15). We found that NDPT was approximately 50- to 60-fold more potent in inhibiting binding of [^{3}H]histamine (K_d 27 μM) than in stimulating cyclic AMP generation ($S_{0.5}$ 1.3 mM) (Fig. 5); that is, NDPT inhibited [^{3}H]histamine binding at concentrations (<0.2 mM) that caused only a small increase in cellular cyclic AMP. To explore further any potential relationship between binding and cyclic AMP, we examined the effect of NDPT on the stimulation of cyclic AMP generation caused by various agonists. We found that NDPT (0.2 mM) (which caused 80%–90% inhibition of [^{3}H]histamine binding) did not alter the response of gastric cells to IMP (Fig. 6A), DPT (Fig. 6B), histamine (Fig. 6C), and 4-MH (Fig, 6D), implying that most of the binding of [^{3}H] histamine occurs to those sites not mediating cyclic AMP generation.

We also examined the effects of two agonists on [^{3}H] histamine binding. IMP was approximately 30-fold more potent in stimulating cyclic AMP generation ($S_{0.5}$ 0.14 μM) than in inhibiting [^{3}H]histamine binding (K_d 4.7 μM) (Figs. 2 and 4). IMP increased cyclic AMP at concentrations that did not inhibit [^{3}H]histamine binding, suggesting that [^{3}H]histamine binds primarily to binding sites that are not linked to adenylate cyclase. Thus, we examined the ability of IMP (1 μM) (which by itself caused

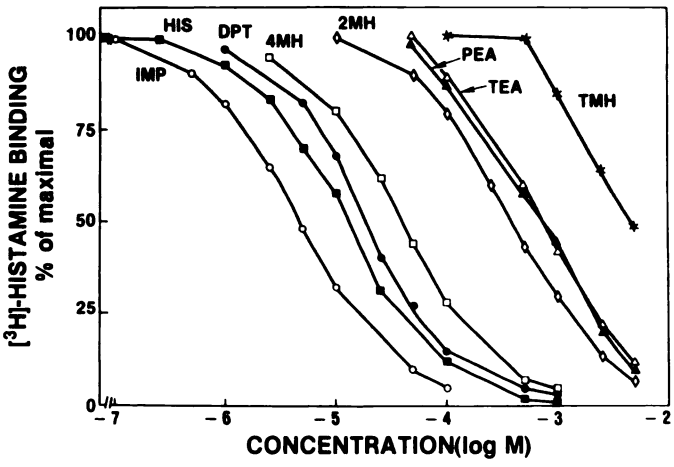

FIG. 4. *Inhibition of [^{3}H]histamine binding by histamine-receptor agonists in dispersed mucosal cells from guinea pig stomach*
Cells were suspended in standard solution containing [^{3}H]histamine (25 nM) and incubated with the indicated agents (*HIS*, histamine). Results are expressed as percentage of the specific binding obtained with the tracer alone (0.10 ± 0.01 pmole/10^6 cells). Each point represents the mean of triplicate determinations; this experiment is representative of four others.

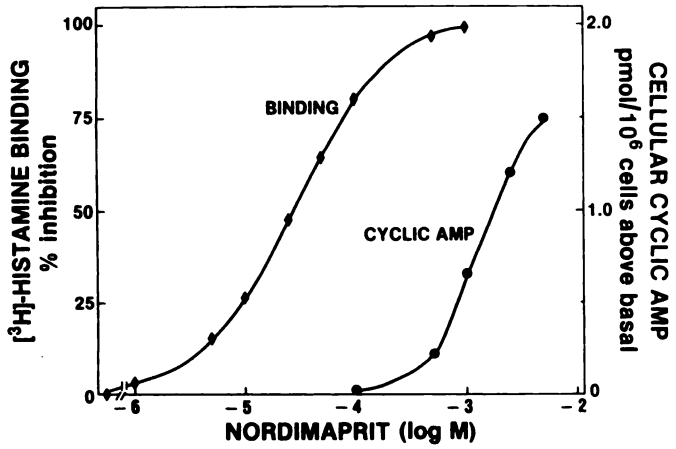

FIG. 5. *Effect of NDPT on cyclic AMP and binding of [^{3}H]histamine in dispersed mucosal cells from guinea pig stomach*

Cellular cyclic AMP and binding were determined as described in legends to Fig. 2 and Fig. 4, respectively. Results for binding are expressed as percentage inhibition of the saturable binding; for cyclic AMP, as the increase above basal (i.e., 0.9 ± 0.15 pmole/million cells). Each point represents the mean of triplicate determinations; this experiment is representative of four others.

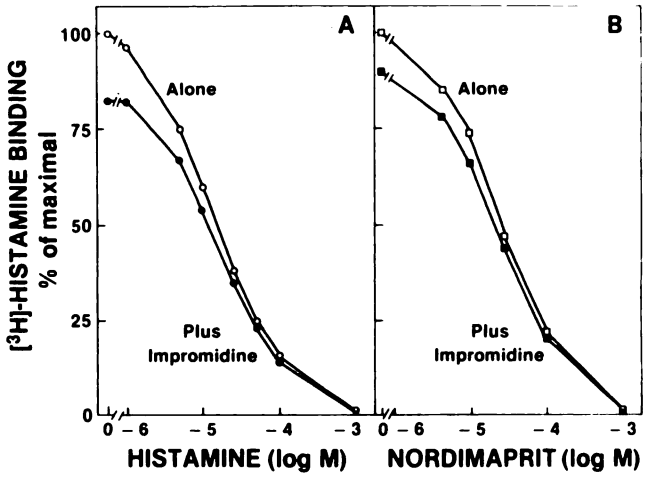

FIG. 7. *Effect of IMP and histamine or NDPT on [^{3}H]histamine binding to dispersed mucosal cells from guinea pig stomach*

Cells were suspended in standard solution containing [^{3}H]histamine (20 nM) with (*closed symbols*) or without (*open symbols*) 1 μM IMP and incubated with the indicated concentrations of histamine (A) or NDPT (B). Results are expressed as percentage of the specific binding obtained with the tracer alone. Each point represents the mean of triplicate determinations; this experiment is representative of two others.

a large increase in cyclic AMP) to alter the inhibition of [^{3}H]histamine binding by other agonists. We found that IMP altered neither the affinity for histamine nor the maximal inhibition of [^{3}H]histamine binding that could be obtained by histamine (Fig. 7A); that is, a concentration of IMP sufficiently high to occupy most of the sites linked to adenylate cyclase did not significantly alter the characteristic of [^{3}H]histamine binding. However, since high concentrations of histamine could effectively compete with IMP on receptor occupation (Fig. 3) and may obscure its potential effect on [^{3}H]histamine binding, we repeated the experiment depicted in Fig. 7A using NDPT

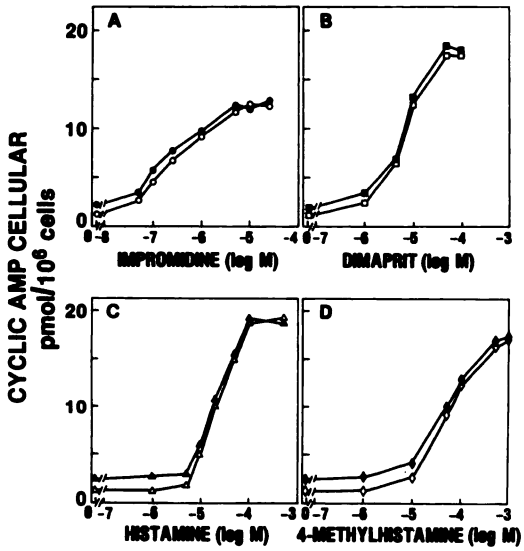

FIG. 6. *Effect of NDPT and various histamine receptor agonists on cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach*

Cells were incubated with (*closed symbols*) or without (*open symbols*) 0.2 mM NDPT plus the indicated concentrations of IMP (A), DPT (B), histamine (C) or 4-MH (D). Each point represents the mean of triplicate determinations.

instead of histamine. NDPT inhibited binding of [^{3}H]histamine at much lower concentrations (K_d 27 μM) than that at which it stimulated cyclic AMP generation ($S_{0.5}$ 1.3 mM) (Fig. 5), and it would be less likely to interfere with IMP in occupation of those sites that are linked to adenylate cyclase. Again, IMP did not alter the affinity for NDPT, and with or without IMP a half-maximal inhibition of tracer binding occurred with 25–30 μM NDPT (Fig. 7B).

Relationship between inhibition of binding and cellular cyclic AMP. Our results with NDPT and IMP suggest that histamine agonists interact with more than one class of binding sites on gastric cells. To examine this possibility we compared the ability of the agonists to inhibit [^{3}H]histamine binding with their ability to increase cyclic AMP. We plotted the percentage of maximal increase in cyclic AMP (Y axis) as a function of the percentage inhibition of tracer binding (X axis) (Fig. 8). We found that with histamine, 4-MH, 2-MH, PEA, and TEA there was a linear relationship between inhibition of tracer binding and cyclic AMP synthesis. Half-maximal stimulation of cyclic AMP synthesis corresponded with 50% inhibition of tracer binding (Fig. 8). In contrast, with IMP, DPT, and NDPT we found this relationship to be nonlinear. The percentage stimulation of cyclic AMP production was best fitted as a hyperbolic function of the percentage inhibition of binding. The calculated parameters indicated that half-maximal stimulation of cyclic AMP synthesis occurred with IMP inhibiting 3.3% and DPT inhibiting 28% of [^{3}H]histamine binding (Fig. 8). With NDPT much greater inhibition of binding was required (90%) before any increase in cyclic AMP could be detected (Fig. 8). Therefore, these results do not support a simple model for the relationship between binding and response.

To analyze these findings further, we compared the

potencies of the agonists by plotting the calculated parameters obtained from cyclic AMP measurements ($S_{0.5}$) as a function of those obtained from the binding studies (K_d) (Table 1). A linear relationship between the two sets of values with a regression line approximating unity would suggest that both binding and stimulation of cyclic AMP synthesis are mediated by a common class of sites and that these sites have equal sensitivity for both processes. However, we found that the relationship between these two sets of values was best fitted by two significantly different regression lines (Fig. 9). The slope of one regression line connecting the values for histamine, 4-MH, 2-MH, PEA, and TEA was 1.04 ± 0.05 (mean ± standard deviation), whereas the second regression line connecting the values for IMP, DPT, histamine, and NDPT was 0.18 ± 0.05. Both lines were significantly different from zero ($r = 0.91$, $p < 0.01$), and the value for histamine was at the intersection between the two regression lines.

DISCUSSION

The present studies showed that the rank order of potencies of the agonists on binding of both [^{3}H]histamine and cellular cyclic AMP were as follows: IMP > DPT > histamine > 4-MH > 2-MH > TEA > PEA. The relative potencies with which these agonists increased cyclic AMP in gastric cells were similar to their relative potencies on functions which are considered to be mediated by H_2 receptors (e.g., gastric acid secretion, contraction of atrial or uterine smooth muscle, and oxygen or [^{14}C]aminopyrine uptake by parietal cells *in vitro*) (3–6, 11, 24). We concluded, therefore, that the actions of these agonists in our system reflect their interaction with H_2 receptors on parietal cells to activate adenylate cyclase and to increase both cyclic AMP and acid formation as reflected by the accumulation of [^{14}C]aminopyrine. We previously found, based on equilibrium (Scatchard) and kinetic studies of [^{3}H]histamine binding, that guinea pig gastric cells possess one class of receptors with no evidence for cooperative interaction between the receptors. Since the increase in cyclic AMP obtained with maximally effective concentrations of histamine plus another agonist was equal to the increase caused by histamine alone, we suggested that these agonists activate a common catalytic moiety of adenylate cyclase (7) by interacting with a single homogeneous class of H_2 receptors (17). However, a single straight line in a Scatchard plot (18) (bound/free versus bound) could be obtained in a system where more than one class of receptors coexist (19).

The present findings do not support our previous interpretation and suggest that guinea pig gastric cells possess more than one class of binding site for the various agonists. Analysis of the relationship between inhibition of tracer binding and increased cellular cyclic AMP revealed the presence of two subgroups of agonists. In one (i.e., histamine, 4-MH, 2-MH, PEA, and TEA) there was a linear relationship between the ability to inhibit tracer

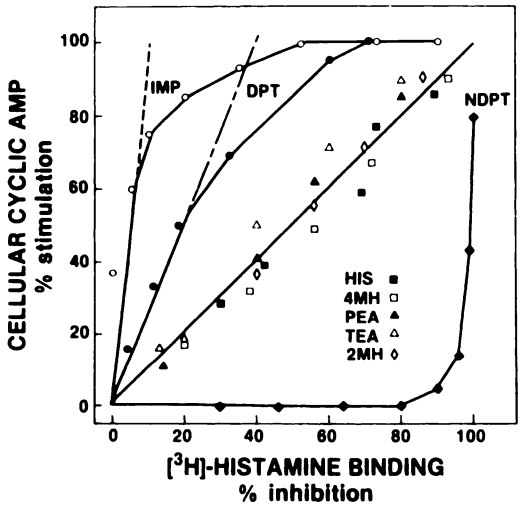

FIG. 8. *Comparison of the ability of histamine-receptor agonists to stimulate increases in cellular cyclic AMP and to inhibit binding of [^{3}H]histamine in dispersed gastric mucosal cells*

Cyclic AMP and binding were determined at the steady state for each process. Values for the increase in cyclic AMP were taken from Fig. 2; those for inhibition of [^{3}H]histamine binding from Fig. 4. The regression line describing histamine (*HIS*), 4-MH, 2-MH, PEA, and TEA was $y = 1.7\ (\pm 6.5) + 1.04\ (\pm 0.05)x$ [correlation coefficient (r) = 0.97]; the intercept was not significantly different from zero ($p > 0.01$), and the slope was not significantly different from unity ($p > 0.01$). The lines describing IMP and DPT were best fitted with the percentage stimulation being a hyperbolic function of the percentage inhibition of ^{3}H-histamine binding. The computer equations were $Y = 103\ (\pm 2.3)/1 + 3.2\ (\pm 0.3)/X$ for IMP and $Y = 120\ (\pm 1.5)/1 + 28.2\ (\pm 0.6)/X$ for DPT, where Y is percentage stimulation of cyclic AMP synthesis and X is percentage inhibition of tracer binding. The line describing NDPT was best fitted with the increase percentage inhibition of binding ($Y1$) being a hyperbolic function of the percentage stimulation of cyclic AMP synthesis ($X1$). The computer equation was $Y1 = 103\ (\pm 2.1)/1 + 2.8\ (\pm 0.2)/X$; that is, when inhibition of tracer binding was inhibited by 50%, the stimulation of cyclic AMP synthesis was 2.8% of maximum. Similar results were obtained from analysis of three other experiments in which cyclic AMP and [^{3}H]histamine binding was measured.

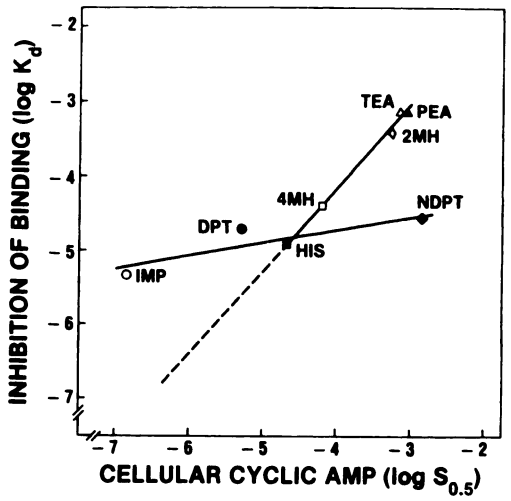

FIG. 9. *Comparison of the parameters that characterize effects of histamine receptor agonists on cellular cyclic AMP and [^{3}H]histamine binding*

Values for the concentrations of agonist required to cause a half-maximal increase in cellular cyclic AMP ($S_{0.5}$) and those required to cause half-maximal inhibition of [^{3}H]histamine binding (K_d) were taken from Table 1. The slope of the regression lines describing histamine (*HIS*), 4-MH, 2-MH, PEA, and TEA was 1.04 ± 0.05 ($r = 0.99$) whereas for IMP, DPT, NDPT, and histamine it was 0.18 ± 0.05 ($r = 0.91$). Both slopes were significantly different from zero ($p < 0.05$).

binding and to increase cyclic AMP, whereas in the other (i.e., IMP, DPT, NDPT) there was a nonlinear relationship between the two processes. The comparison between the K_d values and the $S_{0.5}$ values could also test whether both processes are functionally related to a common binding site. A single regression line between the two sets of values would support the existence of a single class of binding sites mediating both processes. However, our results revealed the presence of two regression lines with significantly different slopes (Fig. 9), suggesting that both processes, i.e., binding and cyclic AMP stimulation, are *not* mediated by a common binding site.

Guinea pig gastric cells appear to possess two distinct binding sites (site A and site B) which are either both parts of the H_2 receptor or only one is the H_2 receptor. Each agonist tested binds to both sites, but only one site (e.g., site A) mediates cyclic AMP generation. The agonists which displayed a linear relationship between their ability to inhibit binding and to increase cyclic AMP bound to both sites with similar potencies. Consequently, the affinity of these sites for the individual agonist was the same whether it was calculated from results on binding or from results on cyclic AMP (Table 1). In contrast, the agonists which displayed a nonlinear relationship with respect to binding and cyclic AMP (i.e., IMP, DPT, and NDPT) also bound to both sites but the affinity of each binding site for the individual agonist was different. Thus, the apparent affinity depended on whether it was calculated from results on binding or from results on cyclic AMP. For instance, the calculated affinity for IMP when measuring cyclic AMP reflected the high affinity between one binding site (e.g., site A) and IMP, because only binding to this site mediates cyclic AMP generation. The calculated affinity when measuring inhibition of binding was determined by the concentrations of agonist required to inhibit [^{3}H]histamine binding. The higher this concentration, the lower the affinity of the binding sites for the agonist. Since with IMP or DPT higher concentrations were required for inhibiting binding than for increasing cyclic AMP, the calculated affinity from binding reflected the low affinity between a second binding sites and these agonists. Therefore, these low-affinity binding sites (e.g., site B) are distinct from the ones mediating cyclic AMP generation, suggesting that gastric cells possess high- and low-affinity binding sites for IMP or DPT and that the high-affinity sites mediate cyclic AMP generation. The two binding sites, however, showed a different pattern of interaction with respect to NDPT. The potency of NDPT for inhibiting [^{3}H]histamine binding was approximately 50-fold higher than for increasing cyclic AMP (Fig. 5). Thus, for NDPT, the high-affinity sites represent those which do not mediate cyclic AMP generation (i.e., site B) whereas the low-affinity sites mediate the biological response. The possibility of gastric cells possessing more than two classes of binding sites (e.g., a high-affinity site plus two classes of low-affinity sites—one for IMP and one for DPT) is unlikely because at sufficiently high concentrations of any agonist binding of [^{3}H]histamine was abolished.

We considered the possibility that the two binding sites are integral parts of the H_2 receptor. However, the observation that the curve describing the effect of IMP on [^{3}H]histamine binding has a single component excludes this possibility and suggests that the high-affinity binding sites constitute only a small fraction of the total binding sites. We also found no evidence for geographical proximity between the binding sites, because occupation of the high-affinity binding site by 1 μM IMP did not affect the affinity of the other site (i.e., site B) for histamine or NDPT (Fig. 7). Furthermore, 0.2 mM NDPT occupied the site not linked to adenylate cyclase (site B), but NDPT did not influence the interaction of IMP, histamine, 4-MH, or DPT with binding site A (Fig. 6). Therefore, these results do not support the hypothesis that one molecule of [^{3}H]histamine binds to both binding sites but suggest that guinea pig gastric cells possess two classes of binding sites for the various histamine agonists. One class constitutes the majority of the binding sites for [^{3}H]histamine and is not linked to adenylate cyclase, whereas the other class mediates the biological response. The later sites probably reflect the H_2 receptors on parietal cells. It should be noted, however, that both classes of binding sites have equal affinity for histamine and therefore are indistinguishable when measuring binding of [^{3}H]histamine with unlabeled histamine. This interpretation might also explain our earlier observations regarding the high binding capacity of gastric cells for histamine (62 pmoles/10^6 cells) (17). It appears from the present data that the majority of this binding is not to the H_2 receptors. This conclusion is also supported by our studies with seven structurally related histamine H_2 antagonists (25). We found that the action of these antagonists on [^{3}H]histamine binding was different from their action on the biological response and was compatible with gastric cells possessing two classes of binding sites for [^{3}H]histamine.

Our present findings agree with previous studies in other preparations in that histamine, 4-MH, and DPT had equal efficacy in stimulating gastric acid secretion (3, 4, 14, 15). However, different species may respond differently and unpredictably to histamine and its analogues. The H_1 agonist PEA (5) was shown to increase [^{14}C]aminopyrine uptake and cyclic AMP in guinea pig gastric cells (7) but failed to do so in a similar preparation from rabbits. Furthermore, DPT, a specific and selective H_2 agonist, stimulated acid secretion in rats (11, 14) but not acid secretion or respiration in isolated frog gastric mucosa (11).

Our results also agree with the rank order of potency of DPT and NDPT for acid secretion *in vivo*. We found that NDPT was 200- to 300-fold less potent than DPT on cellular cyclic AMP whereas *in vivo* NDPT was reported to have 0.001 the activity of dimaprit (14, 15). Our results with IMP contradict studies *in vivo* in which IMP caused an increase in acid secretion comparable in magnitude to that seen with histamine (16). Finally, Lewin *et al.* (26) reported that the dose-response curve for IMP-inhibited [^{14}C]histamine binding to guinea pig gastric cells was to the left of the corresponding curve for activation of adenylate cyclase in sonicated cells. We found that IMP was more potent on cyclic AMP than on binding (Table 1). The basis for this variation is not known, particularly since the potencies of IMP in both preparations were similar (26).

The present studies represent novel findings in that the histamine receptors in guinea pig may prove to be

different from those found in rabbit gastric glands or other mammalian systems. Further comparison between different species could provide information on common characteristics of the histamine receptors, identification of molecular differences between H_1 and H_2 receptors, and more insight into the agonistic and antagonistic compounds with which the H_2 receptors would interact.

ACKNOWLEDGMENTS

We thank Raymond Toles for his technical assistance and Tricia Goforth and Betty Hopper for preparing the manuscript.

REFERENCES

1. Douglas, W. W. Histamine and 5-hydroxytryptamine (serotonin) and their antagonists, in *The Pharmacological Basis of Therapeutics* (A. G. Gilman, L. S. Goodman, and A. Gilman, eds.). Macmillan, New York, 609–646 (1980).
2. Jacobson, E. D., and W. J. Thompason. Cyclic AMP and gastric secretion: the illusive second messenger. *Adv. Cyclic Nucleotide Res.* **7**:199–244 (1976).
3. Black, J. W., W. A. M. Duncan, C. J. Durant, C. R. Ganellin, and M. E. Parsons. Definition and antagonism of histamine H_2-receptors. *Nature* (*Lond.*) **236**:385–390 (1972).
4. Durant, G. J., C. R. Ganellin, and M. E. Parsons. Chemical differentiation of histamine H_1- and H_2-receptor agonists. *J. Med. Chem.* **18**:905–909 (1975).
5. Levi, R., C. R. Ganellin, G. Allan, and H. J. Willens. Selective impairment of atrioventricular conduction by 2-(2-pyridyl)-ethylamine and 2-(2-thiazolyl)-ethylamine, two histamine H_1-receptor agonists. *Eur. J. Pharmacol.* **34**:237–240 (1975).
6. Batzri, S., and J. D. Gardner. Action of histamine on cyclic AMP in guinea pig gastric cells: inhibition by H_1- and H_2-receptor antagonists. *Mol. Pharmacol.* **16**:406–416 (1979).
7. Batzri, S., and J. Dyer. Aminopyrine uptake by guinea pig gastric mucosal cells: mediation by cyclic AMP and interaction among secretagogues. *Biochim. Biophys. Acta* **675**:416–426 (1981).
8. Scholes, P., A. Cooper, D. Jones, J. Major, M. Walters, and C. Wilde. Characterization of an adenylate cyclase system sensitive to histamine H_2-receptor excitation in cells from dog gastric mucosa. *Agents Actions* **6**:677–682 (1976).
9. Soll, A. H., and A. Wollin. Histamine and cyclic AMP in isolated canine parietal cells. *Am. J. Physiol.* **237**:E444–E450 (1979).
10. Wollin, A., A. H. Soll, and I. M. Samloff. The action of histamine secretion and PGE_2 on cyclic AMP production by isolated canine fundic mucosal cells. *Am. J. Physiol.* **237**:E437–E443 (1979).
11. Chew, C. S., and S. J. Hersey. Characteristics of histamine receptor in isolated gastric glands, in *Hormone Receptors in Digestion and Nutrition* (G. Roselin, P. Fromageot, and S. Bonfils, eds.). Elsevier Biomedical Press, Amsterdam, 361–372 (1972).
12. Sonnenberg, A., W. Hunziker, H. R. Koelz, J. A. Fischer, and A. L. Blum. Stimulation of endogenous cyclic AMP (cAMP) in isolated cells by histamine and prostaglandin. *Acta Physiol. Scand.* [*Suppl.*] 307–317 (1978).
13. Sonnenberg, A., T. Berglindh, M. J. M. Lewin, J. A. Fischer, G. Sachs, and A. L. Blum. Stimulation of acid secretion in isolated gastric cells, in *Hormone Receptors in Digestion and Nutrition* (G. Roeselin, P. Fromageot, and S. Bonfils, eds.). Elsevier Biomedical Press, Amsterdam, 337–348 (1979).
14. Parsons, M. E., D. A. A. Owen, C. R. Ganellin, and G. J. Durant. Dimaprit—[*S*-[3-(*N*,*N*-dimethylamino)propyl]isothiourea]—a highly specific histamine H_2-receptor agonist. Part I. Pharmacology. *Agents Actions* **7**:31–37 (1977).
15. Durant, G. J., C. R. Ganellin, and M. E. Parsons. Dimaprit—[*S*-[3-(*N*,*N*-dimethylamino)propyl]isothiourea]—a highly specific histamine H_2-receptor agonist. Part 2. Structure-activity considerations. *Agents Actions* **7**:39–43 (1977).
16. Durant, G. J., W. A. M. Duncan, C. R. Ganellin, M. E. Parsons, R. C. Blakemore, and A. C. Rasmussen. Impromidine (SK&F 92676) is a very potent and specific agonist for histamine H_2-receptors. *Nature* (*Lond.*) **276**:403–405 (1978).
17. Batzri, S. Interaction of histamine with specific membrane receptors on gastric mucosal cells. *Biochem. Pharmacol.* **30**:3013–3016 (1981).
18. Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**:660–672 (1948).
19. Birnbaumer, L., S. L. Pahl, and A. J. Kaumann. Receptors and acceptors: a necessary distinction in hormone binding studies. *Adv. Cyclic Nucleotide Res.* **4**:239–281 (1974).
20. Batzri, S., and J. D. Gardner. Potassium transport in dispersed mucosal cells from guinea pig stomach. *Biochim. Biophys. Acta* **508**:328–338 (1978).
21. Batzri, S., and J. D. Gardner. Cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach. *Biochim. Biophys. Acta* **541**:181–189 (1978).
22. Akera, T., and V. K. Cheng. A simple method for the determination of affinity and binding site concentration in receptor binding studies. *Biochim. Biophys. Acta* **470**:412–423 (1977).
23. Boeynaems, J. M., and J. E. Dumont. Coupling between ligand binding and receptor response, in *Outlines of Receptor Theory*. Elsevier Biomedical Press, Amsterdam, 123–150 (1980).
24. Berglindh, T., H. Helander, and G. Sachs. Secretion at the parietal cell level—a look at rabbit gastric glands. *Scand. J. Gastroenterol.* [*Suppl.*] **55**:7–14 (1979).
25. Batzri, S., J. W. Harmon, J. Dyer, and W. F. Thompson. Interaction of histamine with gastric mucosal cells: effect of histamine H_2 antagonists on binding and biological response. *Mol. Pharmacol.* **22**:41–47 (1982).
26. Lewin, M. J. M., F. Grelac, A. M. Cheret, E. Rene, and S. Bonfils. Demonstration and characterization of histamine H_2-receptor on isolated guinea pig gastric cell, in *Hormone Receptors in Digestion and Nutrition* (G. Roeselin, P. Fromageot, and S. Bonfils, eds.). Elsevier Biomedical Press, Amsterdam, 383–390 (1979).

Send reprint requests to: Dr. Shmuel Batzri, Uniformed Services University of the Health Sciences, Room A3017, 4301 Jones Bridge Road, Bethesda, Md. 20014.