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Action of Histamine on Cyclic AMP in Guinea Pig Gastric Cells: Inhibition by H_1- and H_2-Receptor Antagonists

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SUMMARY

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In dispersed cells from guinea pig fundic mucosa cyclic AMP was increased 6- to 13-fold by histamine as well as each of 3 chemically related analogues. The relative potencies of these agonists were histamine > 4-methylhistamine > 2-methylhistamine > 2-(2-pyridyl)ethylamine and the relative efficacies of these agents were histamine = 4-methylhistamine > 2-methylhistamine > 2-(2-pyridyl)ethylamine. The increase in cellular cyclic AMP caused by maximally effective concentrations of two agonists in combination was equal to the increase caused by the more effective agonist alone. The increase in cyclic AMP caused by each agonist could be inhibited competitively by metiamide or cimetidine as well as by promethazine or diphenhydramine. The inhibitory potencies of metiamide and cimetidine were greater than those of promethazine and diphenhydramine. Sufficiently high concentrations of each antagonist abolished the action of each agonist and sufficiently high concentrations of each agonist could overcome the inhibition caused by each antagonist. For a given antagonist the inhibitory potency calculated from results with histamine was not significantly different from that calculated from results with each of the other agonists. 2-(2-pyridyl)ethylamine was a partial agonist and its potency as an agonist was equal to its potency as an inhibitor of the action of histamine. These results indicate that in dispersed cells from guinea pig fundic mucosa the increase in cyclic AMP caused by histamine or its 3 analogues can be inhibited competitively by metiamide or cimetidine as well as by classical antihistamines.

INTRODUCTION

Actions of histamine are thought to reflect its interaction with one of two classes of receptors. Those actions which are mediated by H_1-receptors (e.g., contraction of smooth muscle from intestine or bronchus) can be inhibited competitively by low concentrations of classical antihistamines (1, 2). Those actions which are mediated by H_2-receptors (e.g., gastric acid secretion or contraction of atrial or uterine smooth muscle) can be inhibited competitively by relatively low concentrations of so-called H_2-receptor antagonists such as burimamide, metiamide and cimetidine (3–5). Furthermore, 2MH[1] and PEA are more potent than 4MH in causing effects mediated by H_1-receptors while the reverse is true for actions mediated by H_2-receptors (6, 7).

Histamine increases cyclic AMP in gastric mucosa, and activates adenylate cy-

[1] The abbreviations used are: 2MH, 2-methylhistamine; PEA, 2-(2-pyridyl)ethylamine; 4MH, 4-methylhistamine; AMP, adenosine 3′:5′-monophosphate.

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clase in broken cell preparations from several tissues (8–16; for review see ref. 17, 18). In dispersed gastric mucosal cells (8) and in homogenates of brain, heart or gastric mucosa (9–12, 16), the action of histamine can be inhibited by classical antihistamines and by burimamide or metiamide. In dispersed mucosal cells from guinea pig stomach, this inhibition was specific for histamine in that the increase in cyclic AMP caused by theophylline or by PGE_1 was not altered by diphenhydramine or metiamide (8).

To examine further the abilities of histamine antagonists to inhibit the actions of histamine on dispersed gastric mucosal cells we have measured the increase in cellular cyclic AMP caused by histamine and 3 chemically related analogues as well as the abilities of various H_1- and H_2-receptor antagonists to inhibit these actions. Our results indicate that in dispersed mucosal cells from guinea pig stomach, the increase in cyclic AMP caused by histamine or each of 3 analogues can be inhibited competitively by metiamide or cimetidine as well as by classical antihistamines.

MATERIALS

Male Hartley albino guinea pigs (200–250 g) were obtained from the Small Animal Section, Veterinary Resources Branch, National Institutes of Health, Bethesda, Maryland. [^{125}I] succinyl cyclic AMP tyrosine methyl ester (>150 Ci/mmole) and cyclic AMP antiserum (prepared with a second antibody) were from New England Nuclear, Boston, Mass; bovine serum albumin from Armour; Nylon mesh screen (No. 114T) from Nytex; theophylline, crude collagenase and cyclic AMP from Sigma Chemical Co., St. Louis, Mo.; histamine from Calbiochem, Los Angeles, Cal.; promethazine from Wyeth Laboratories, Philadelphia, Penn.; and diphenhydramine from Parke Davis Co., Detroit, Mich. Metiamide, cimetidine, 2MH, 4MH and PEA were gifts from Smith, Kline and French Laboratories, Philadelphia, Penn. Hank's buffer was from GIBCO or was prepared in our laboratory. The standard incubation solution was a modification of Hank's 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, 5.55 mM glucose, 2.0 mM glutamine, 0.001% (w/v) phenol red, BME vitamin solution (GIBCO), 15 mM $NaHCO_3$ and 15 mM Hepes (pH 7.4).

METHODS

Dispersed gastric cells from guinea pig stomach were prepared as described previously (19). Dispersed cells were collected by centrifugation, washed twice with iced, standard incubation solution and then washed once with and resuspended in standard incubation solution containing 5 mM theophylline. The suspension of gastric cells contained 70–80% parietal cells with the remainder being mucous, chief and undifferentiated cells that could not be identified. For ultrastructural analysis the cell suspension was fixed with 2.5% (v/v) glutaraldehyde (in Hank's buffer) for 1 hour at 4°. The fixed cell suspension was washed 3 times with Hank's buffer and postfixed by incubation with 1% (w/v) osmium tetroxide for 45 minutes at 4°. The fixed cells were dehydrated with a graded series of ethanol solutions, embedded in Epon 812, sectioned, stained with uranyl acetate and lead citrate, and examined with a Siemens model 101 electron microscope at 80 kV. Six hundred cells were examined in electron micrographs from a representative cell suspension. From the morphologic characteristics of these cells, 73% were parietal cells, 12% were mucous cells, 9% were chief cells and 6% could not be identified. No cells possessing the morphological characteristics of mast cells or enterochromaffin cells could be identified; however, the conditions used to prepare the cell suspension for examination may not have been optimal for preserving the ultrastructural characteristics of mast cells or enterochromaffin cells. When incubated with histamine (1 mM) or carbachol (1 mM), the parietal cells showed characteristic morphological changes in the tubulovesicular system similar to those observed in intact gastric mucosa (20). Greater than 95% of the cells excluded trypan blue and incubating the cells for 60 minutes at 37° with histamine as well as various analogues or antagonists did not alter the percentage of cells which excluded

trypan blue. Furthermore, none of the agents tested in the present studies altered the cellular content of sodium or potassium during a 2-hour incubation at 37° (19).

Cellular cyclic AMP was determined by radioimmunoassay as described previously (8). As we have reported (8), theophylline increases cyclic AMP in dispersed gastric cells, augments the increase in cyclic AMP caused by histamine or prostaglandin E_1 but does not alter the time course of the increase in cyclic AMP caused by a given agent. Since with all agents tested cellular cyclic AMP became constant after 15–20 minutes, all incubations contained 0.5 ml cell suspension plus the appropriate agents and were for 20 minutes at 37°. In control cells and in cells incubated with various agents cyclic AMP was linear function of cell concentration from 0.5 million to 10 million cells/ml. The following compounds were tested at at least 10 pmol per assay tube (an amount at least 20-times greater than the maximal amount of cyclic AMP measured under any incubation condition in the present studies) and were found not to interfere with the assay for cyclic AMP: cyclic GMP, ATP, ADP, AMP, adenosine, adenine, GTP, GDP, GMP, guanosine, guanine, ITP, UTP, CTP and inositol phosphate. The immunoreactive cyclic AMP was abolished by incubation with beef heart cyclic nucleotide phosphodiesterase and co-chromatographed with native cyclic AMP (using the chromatographic system described by Salomon et al. (21) and used previously by us in other studies (22)). Recovery of added cyclic AMP was 97 ± 8% (mean of 16 determinations ± 1 SD). None of the agents tested in the present study interfered with the radioimmunoassay of cyclic AMP.

RESULTS

Experimental observations. Cyclic AMP in dispersed mucosal cells from guinea pig stomach was increased by histamine as well as by each of 3 analogues - 4MH, 2MH and PEA (Fig. 1). An increase in cellular cyclic AMP could be detected with 2 μM histamine, was half-maximal at 30 μM and was maximal at 1 mM (Fig. 1). If the potency[2] of histamine is assigned a value of 100, the relative potencies of the analogues were 4MH, 50; 2MH, 6; and PEA, 3. At maximally effective concentrations histamine and 4MH each caused a 13-fold increase in cellular cyclic AMP while the increase with 2MH was 10-fold and with PEA was 6-fold (Fig. 1). To compare the response to two agonists in combination with that to each agonist alone, we measured cyclic AMP in dispersed mucosal cells incubated with maximally effective concentrations of each agonist alone and in combination with another agonist. The increase in cellular cyclic AMP caused by maximally effective concentrations of two agents in combination was the same as the increase caused by the more effective agent alone (Table 1). For example, the increase in cyclic AMP caused by histamine plus one of the analogues was the same as that caused by histamine alone (Table 1).

Metiamide or promethazine did not alter cellular cyclic AMP but each agent inhibited the increase in cyclic AMP caused by histamine or its various analogues (Fig. 2). At relatively low concentrations of agonist the increase in cyclic AMP could be abolished by metiamide or by promethazine (Fig. 2). With histamine or 4MH the inhibition caused by metiamide as well as that caused by promethazine was progressively reduced and eventually abolished by increasing the concentration of the agonist (Fig. 2A and 2B). With 2MH or PEA the inhibition caused by metiamide or promethazine was reduced progressively but not abolished by increasing the concentration of agonist (Fig. 2C and 2D). Since our supplies were limited we were not able to test 2MH and PEA at concentrations above 5 mM and 10 mM, respectively. Results similar to those illustrated in Fig. 2 were obtained using cimetidine or diphenhydramine instead of metiamide or promethazine.

The actions of metiamide and promethazine were investigated further by examining the ability of various concentrations of these antagonists to inhibit the increase in

[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.

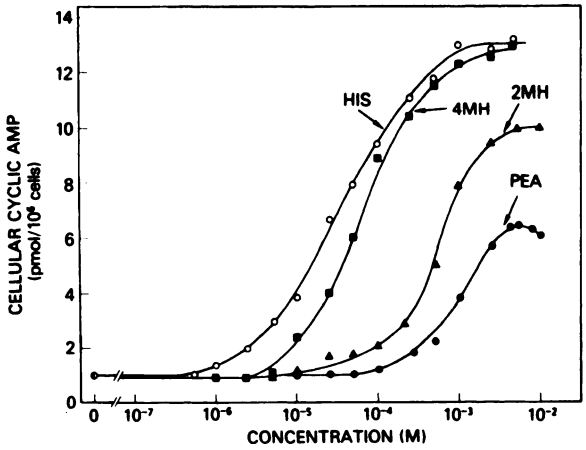

FIG. 1. *Effect of histamine and its analogues on cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach*

Cells were suspended in standard incubation solution and incubated with histamine (HIS), 4-methylhistamine (4MH), 2-methylhistamine (2MH) or 2-(2-pyridyl)ethylamine (PEA). Each point is the mean of triplicate determinations and this experiment is representative of six others.

cellular cyclic AMP caused by two different concentrations of histamine or one of its analogues (Figs. 3 and 4). The increase in cellular cyclic AMP caused by 40 μM histamine was reduced detectably with 0.3 μM metiamide, by 50% with 3 μM metiamide and was abolished with 100 μM metiamide (Fig. 3A). With a higher concentration of histamine (100 μM) higher concentrations of metiamide were required to cause inhibition comparable in magnitude to that seen with 40 μM histamine (Fig. 3A). A similar pattern of action of metiamide was seen using two different concentrations of 4MH (Fig. 3B), 2MH (Fig. 3C) or PEA (Fig. 3D). That is, increasing the concentration of agonist increased the concentration of metiamide required to produce 50% inhibition. Sufficiently high concentrations of metiamide, however, were able to abolish the action of both concentrations of agonist. Results similar to those illustrated in Fig. 3 were obtained using promethazine instead of metiamide (Fig. 4). With histamine (Fig. 4A), 4MH (Fig. 4B), 2MH (Fig. 4C) and PEA (Fig. 4D) higher concentrations of agonist resulted in higher concentrations of promethazine being required to produce 50% inhibition. The action of each concentration of each agonist tested could be abolished by sufficiently high concentrations of promethazine. Results similar to those illustrated in Fig. 3 were obtained using cimetidine instead of metiamide and results similar to those illustrated in Fig. 4 were obtained using diphenhydrine instead of promethazine (not shown).

TABLE 1

Effect of histamine and its analogs on cyclic AMP in mucosal cells from guinea pig stomach

Cells were suspended in standard incubation solution containing the indicated agents and incubated for 20 min at 37°. Each value is the mean (±1 SD) of triplicate determinations and this experiment is representative of four others.

Agent	Cellular cyclic AMP
	(pmol/10⁶ cells)
None	1.1 ± 0.2
Histamine (1 mM)	16 ± 2.0
4-Methylhistamine (1 mM)	15 ± 2.1
plus histamine	16 ± 2.2
2-Methylhistamine (5 mM)	10 ± 1.5
plus histamine	15 ± 1.6
plus 4-methylhistamine	14 ± 2.0
2-Pyridylethylamine (5 mM)	7.5 ± 1.5
plus histamine	17 ± 1.8
plus 4-methylhistamine	15 ± 1.9
plus 2-methylhistamine	11 ± 2.0

If PEA is a partial agonist with respect to histamine, PEA should behave as competitive antagonist of the action of histamine on cyclic AMP. When these two agonists were tested in combination, PEA in-

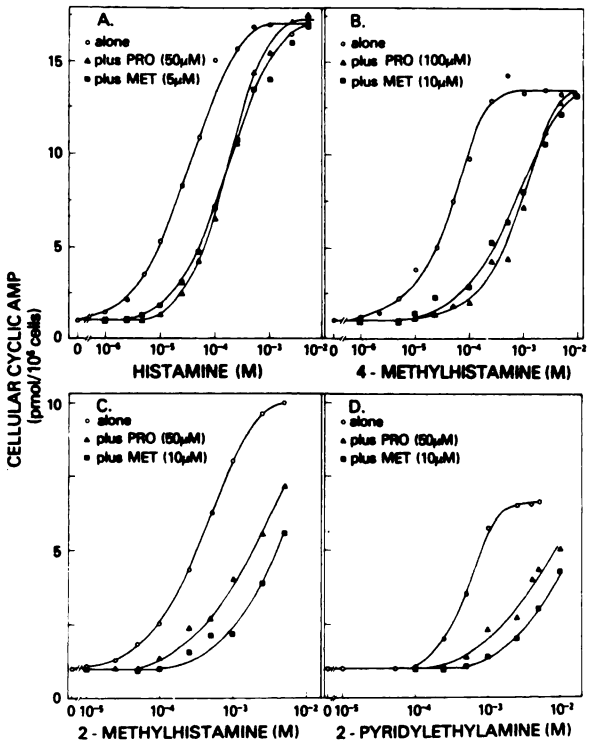

FIG. 2. *Effect of histamine receptor agonists and antagonist on cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach*

Cells were incubated with varying concentrations of histamine (panel A), 4-methylhistamine (panel B), 2-methylhistamine (panel C) or 2-(2-pyridyl)ethylamine (panel D) with or without the indicated concentration of promethazine (PRO) or metiamide (MET). Each point is the mean of triplicate determinations and this experiment is representative of three others.

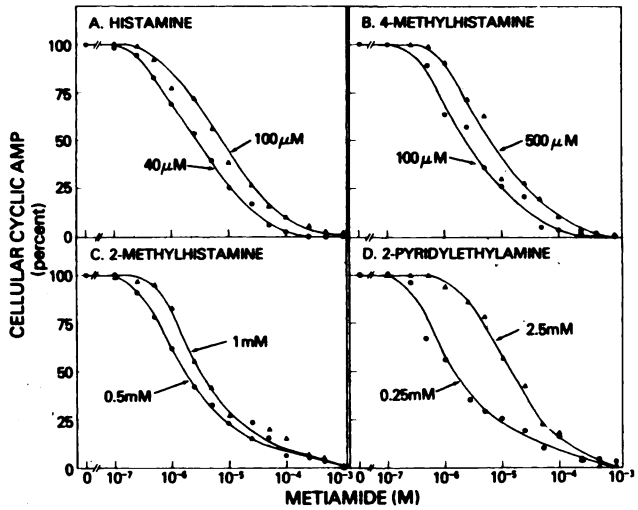

FIG. 3. *Effect of metiamide on cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach*

Cells were incubated with varying concentrations of metiamide plus two different concentrations of histamine or one of three analogues. Each point is the mean of triplicate determinations and this experiment is representative of three others.

hibited the action of histamine (Fig. 5). In the absence of PEA, an increase in cellular cyclic AMP could be detected with 2 μM histamine and was half-maximal at 30 μM histamine (Figs. 1, 2 and 5A). In the presence of 2.5 mM PEA (which caused a 5-fold increase in cyclic AMP), 10 μM histamine was required to produce a detectable effect and 110 μM histamine was required to produce a half-maximal effect (Fig. 5).

Mathematical analysis of results. Results obtained with each of the four agonists tested were compatible with the agonist interacting with a single class of homogeneous receptors. The increase in cellular cyclic AMP caused by histamine, 4MH, 2MH or PEA alone (Figs. 1 and 2) or in combination with a constant concentration of metiamide or promethazine (Fig. 2) could be described by the equation $R = R_{max}(A/(A + S_{0.5})$ where R is the observed increase in cyclic AMP, A is the concentration of

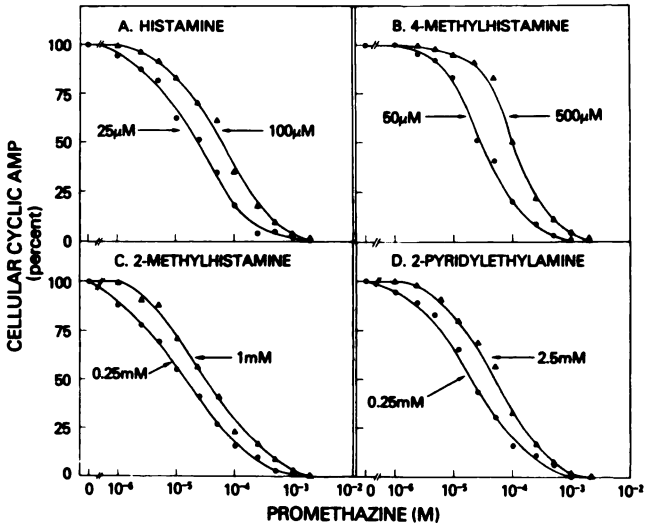

FIG. 4. *Effect of promethazine on cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach*

Cells were incubated with varying concentrations of promethazine plus two different concentrations of histamine or one of three analogues. Each point is the mean of triplicate determinations and this experiment is representative of three others.

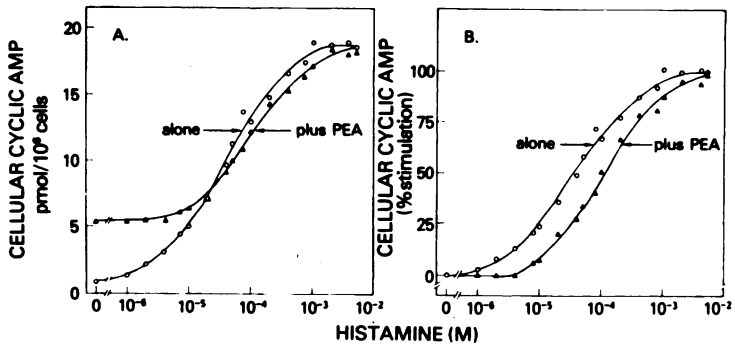

FIG. 5. *Effect of histamine and 2-(2-pyridyl)ethylamine (PEA) on cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach*

In panel A, cells were incubated with or without 2.5 mM PEA plus the indicated concentration of histamine. In panel B, cellular cyclic AMP is expressed as % of the maximal increase caused by histamine. Each point is the mean of triplicate determinations and this experiment is representative of three others.

agonist, R_{max} is the increase in cyclic AMP caused by maximally effective concentration of agonist and $S_{0.5}$ is the concentration of agonist required to produce a half-maximal response. In each experiment, R_{max} and $S_{0.5}$ were calculated from experimentally determined values. Although with a given agonist, values for R_{max} (whether computed or measured directly) differed significantly from one experiment to another, the values for $S_{0.5}$ did not. For example, in three experiments using histamine the values for R_{max} (pmol cyclic AMP/10^6 cells) were 13.3 ± 1.4, 19.6 ± 1.8 and 15.6 ± 1.7 while the corresponding values for $S_{0.5}$ (μM) were 29 ± 3, 31 ± 5 and 32 ± 4. Similar results were obtained with 4MH, 2MH and PEA.

The results obtained using metiamide and promethazine as well as other histamine antagonists suggested that these agents were functioning as competitive antagonists in our system. To evaluate this possibility further we analyzed our results using the equation $K_I = (F/1\text{-}F)\ (B \cdot S_{0.5}/(A + S_{0.5})$ where K_I is the concentration of antagonist required to occupy 50% of the receptors in the absence of agonist, F is the observed increase in cellular cyclic AMP expressed as the fraction of that obtained without the antagonist, and B is the concentration of the antagonist. The value of K_I was computed using various transformations of this equation depending on whether varying concentrations of agonist were tested with a constant concentration of antagonist or *vice versa* (Table 2).

The value of K_I for metiamide tested with one concentration of agonist was not significantly different from the value obtained when metiamide was tested with a second concentration of the same agonist. For example, with 40 μM histamine the value of K_I for metiamide was 1.3 ± 0.4 μM and with 100 μM histamine the value was 1.9 ± 0.4 μM. This was also the case when values of K_I for metiamide were calculated from results obtained using two different concentrations of 4MH, 2MH or PEA. With a given agonist the value of K_I for metiamide was the same whether it was calculated from experiments with varying concentrations of metiamide (Fig. 3) or from experiments with varying concentrations of agonist (Fig. 2). Finally, the value of K_I for metiamide calculated from results with one agonist (e.g., histamine) was not significantly different from the value calculated from results with each of the other agonists (e.g., 4MH, 2MH or PEA) (Table 2). There was also close agreement between the various values of K_I for promethazine calculated from experiments performed using promethazine instead of metiamide (Table 2).

The value of $S_{0.5}$ for PEA calculated from results obtained with PEA alone (Table 2) was 900 ± 300 μM (mean ± 1 SD from three experiments). This value was not significantly different from the value of K_I for PEA calculated from results obtained when PEA acted as competitive antagonist of the action of histamine (i.e., 950 ± 240) (Fig. 5).

TABLE 2

Values of parameters that characterize effects of histamine receptor agonists and antagonists on cyclic AMP on dispersed mucosal cells from guinea pig stomach

Agonist tested	$S_{0.5}$[a]	R_{max}[b]	K_I[c]	
			Metiamide	Promethazine
	(μM)		(μM)	(μM)
Histamine	29 ± 7 (15)	100	1.4 ± 0.4 (9)	13 ± 2 (9)
4-methylhistamine	60 ± 15 (9)	95 ± 9 (9)	0.9 ± 0.3 (8)	10 ± 4 (8)
2-methylhistamine	500 ± 150 (6)	77 ± 6 (9)	0.9 ± 0.6 (6)	11 ± 2 (6)
2-pyridylethylamine	900 ± 300 (6)	50 ± 8 (9)	1.5 ± 0.5 (6)	13 ± 4 (6)

[a] $S_{0.5}$ is the concentration of agonist required to cause a half-maximal increase in cellular cyclic AMP.

[b] R_{max} is the increase in cellular cyclic AMP caused by a maximally effective concentration of agonist and is expressed as a percent of the value for histamine.

[c] K_I is the concentration of antagonist required to occupy 50% of the receptors in the absence of agonist and results are given for each of the agonists tested. All values are means ± 1 SD from the number of experiments given in parentheses.

Additional analyses were performed using results not given in the present paper. The values of K_I calculated from experiments using cimetidine or diphenhydramine showed the same pattern of agreement that we obtained with metiamide or promethazine. We also measured the increase in cellular cyclic AMP caused by 4MH alone or with four different concentrations of promethazine. When the results were analyzed as described by Arunlakshana and Schild (23) we found that increasing concentrations of promethazine caused a parallel displacement of the dose-response curve for the increase in cellular cyclic AMP caused by 4MH. The slope of the line relating log (DR-1) to log (promethazine) was not significantly different from unity and the pK_B was 4.85 (K_I, 14 μM).

DISCUSSION

In the present studies we found that histamine and 3 chemically related agonists could cause a 6- to 13-fold increase in cyclic AMP in dispersed mucosal cells prepared from guinea pig stomach. The relative potencies of the agonists were histamine > 4MH > 2MH > PEA and their relative efficacies were histamine = 4MH > 2MH > PEA. The increase in cyclic AMP caused by each agonist could be inhibited competitively by classical antihistamines (promethazine and diphenhydramine) as well as by H_2-receptor antagonists (metiamide and cimetidine). Although we found that H_2-receptor antagonists could competitively inhibit the action of histamine, several findings indicate that the action of histamine in our system reflects its interaction with H_2-receptors. In inhibiting the action of histamine and related agonists, metiamide and cimetidine were approximately 10-times more potent (K_I, 1 μM) than promethazine or diphenhydramine (K_I, 10 μM). Furthermore, the potency with which metiamide or cimetidine inhibited the histamine-induced increase in cyclic AMP in gastric mucosal cells was similar to the potency with which these antagonists inhibit the action of histamine on other functions which are generally considered to be mediated by H_2-receptors (e.g., gastric acid secretion and contraction of atrial or uterine smooth muscle (3–5)). In contrast, the potency with which promethazine or diphenhydramine inhibited the histamine-induced increase in cyclic AMP in gastric mucosal cells was approximately 1000-times less than the potency with which these antagonists inhibit the action of histamine on other functions which are generally considered to be mediated by H_1-receptors (e.g., contraction of guinea pig ileum (1, 2)). Finally, as in other systems in which the action of histamine is mediated by H_2-receptor (6, 7), we found that 4MH was a more potent agonist than was 2MH or PEA.

Our present results agree with previous studies of the action of histamine on cyclic AMP or adenylate cyclase in gastric mucosa (8–11) as well as in other tissues (12–16) in that the histamine-induced increase in adenylate cyclase activity could be inhibited by H_1- as well as by H_2-receptor antagonists. In homogenates of brain (13–16) or cardiac muscle (12) the increase in adenylate cyclase activity caused by histamine or its methylated derivatives could be inhibited competitively by H_1- as well as by H_2-receptor antagonists and the inhibitory potency of the H_2-receptor antagonists was approximately 10-times greater than that of the H_1-receptor antagonists (12, 16). In some studies using homogenates of gastric mucosa or dispersed gastric mucosal cells (10, 11, 24), H_1-receptor antagonists have been shown to inhibit the action of histamine on adenylate cyclase or cellular cyclic AMP in a noncompetitive fashion and, in addition, to inhibit the activation of adenylate cyclase caused by sodium fluoride or by prostaglandins. The action of histamine on contraction of atrial or uterine muscle or on acid secretion from intact gastric mucosa has not been inhibited by H_1-receptor antagonists (3).

In the preparation used for the present studies (containing 70–80% parietal cells) histamine as well as PGE_1 can cause a significant increase in cellular cyclic AMP and the increase in cyclic AMP caused by histamine plus PGE_1 is equal to the sum of the increase caused by each agonist alone (8). In dispersed mucosal cells prepared

from dog gastric mucosa, histamine and various prostaglandins have also been found to increase cyclic AMP (25). In dog gastric mucosal cells an increase in the percentage of parietal cells was accompanied by an increase in the magnitude of histamine-stimulated cyclic AMP and a corresponding reduction in the magnitude of prostaglandin-stimulated cyclic AMP. Furthermore, the actions of histamine and prostaglandins on cyclic AMP were not additive. Instead, prostaglandins inhibited, but did not abolish, the histamine-induced increase in cyclic AMP and this inhibition occurred with concentrations of prostaglandins that were substantially lower than those required to increase cyclic AMP. We cannot account for the lack of effect of prostaglandins on the histamine-induced increase in cyclic AMP in guinea pig gastric mucosal cells; however, in homogenates of guinea pig gastric mucosa the actions of prostaglandins on adenylate cyclase activity are also additive with those of histamine (26).

Although classical antihistamines have been shown to inhibit the action of histamine on adenylate cyclase in gastric mucosa, these antagonists have usually not altered the action of histamine on gastric acid secretion (2). It is possible that cyclic AMP does not mediate the action of histamine on gastric acid secretion. This possibility seems unlikely since histamine has been shown to increase cyclic AMP in parietal cells (25), and there is a reasonably close correlation between the ability of histamine to increase cyclic AMP in dispersed mucosal cells from dog gastric mucosa and its ability to alter functions which are thought to reflect acid secretion (e.g., oxygen consumption and tissue accumulation of aminopyrine (27, 28)). It is also possible that in intact gastric mucosa H_1-receptor antagonists, at the concentrations used, may not be able to gain access to the receptors with which histamine interacts to increase acid secretion. Evidence in favor of this possibility is the finding that tripellenamine, a classical antihistamine, can inhibit the action of histamine on gastric acid secretion when the antagonist is injected intraarterially but not when it is given intravenously (29). Finally, it is possible that in preparing the tissue for *in vitro* studies it is altered in such a way that the action of histamine can be inhibited by classical antihistamines.

Although our present findings show that the increase in cyclic AMP caused by histamine and chemically-related agonists can be inhibited competitively by H_1- as well as by H_2-receptor antagonists, we cannot determine whether this inhibition is of the *fully* competitive type or of the *partially* competitive type (30). From the mechanistic standpoint the simplest explanation for our present findings is that each antagonist is a *fully* competitive inhibitor. That is, histamine increases cyclic AMP in our preparation by interacting with a single class of receptors which can also interact with H_1-receptor antagonists and with H_2-receptor antagonists. On the other hand, our results are equally compatible with the possibility that one or both classes of histamine antagonists are functioning as *partial* competitive antagonists in that they interact with a site which is functionally distinct from the histamine receptor and by so doing reduce the affinity of the histamine receptor for histamine and chemically related agonists. The hypothesis that classical antihistamines are acting as *partial* competitive antagonists has the advantage of offering a possible explanation for the variable effects of H_1-receptor antagonists on actions of histamine thought to be mediated by H_2-receptors. That is, although metiamide and cimetidine may inhibit the interaction of histamine with H_2-receptors in a *fully* competitive fashion, classical antihistamines may alter these actions of histamine by interacting with a site which is functionally distinct from the histamine receptor. In some tissues this interaction may reduce the potency but not the efficacy of histamine (i.e., *partial* competitive antagonism) while in other tissues, this interaction may reduce the efficacy but not the potency of histamine (i.e., noncompetitive antagonism).

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