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Interaction of Histamine with Gastric Mucosal Cells

Effect of Histamine H_2 Antagonists on Binding and Biological Response

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SUMMARY

In dispersed mucosal cells from guinea pig stomach, cimetidine as well as each of six chemically related H_2 antagonists inhibited binding of [^{3}H]histamine and the histamine-stimulated increase in cellular cyclic AMP. The inhibition by these antagonists of cyclic AMP stimulation was competitive with respect to histamine, and the K_i values were as follows: tiotidine, 0.04 ± 0.02 μM; ranitidine, 0.15 ± 0.06 μM; etintidine (BL 5641A), 0.22 ± 0.06 μM; cimetidine, 0.81 ± 0.20 μM; SKF 92629, 0.93 ± 0.27 μM; metiamide, 1.41 ± 0.41 μM, and SKF 92408, 3.05 ± 1.02 μM. In inhibiting [^{3}H]histamine binding, the *relative* as well as the absolute potencies of these antagonists were different from their potencies on inhibiting cyclic AMP stimulation. Higher concentrations of H_2 antagonists were required to inhibit [^{3}H]histamine binding than to inhibit cyclic AMP stimulation. Sufficiently high concentrations of each antagonist abolished [^{3}H]histamine binding to gastric cells. Neither cimetidine nor histamine altered the rate of dissociation of bound [^{3}H]histamine from gastric cells. Performing a direct comparison by plotting the log [potency] for inhibition of binding (i.e., K_d) against the log [potency] for inhibiting cyclic AMP stimulation caused by histamine (i.e., K_i) revealed that there was no correlation between the two sets of values, and the slope of the regression line was not significantly different from zero. These results agree with our earlier findings with histamine receptor agonists and are compatible with the hypothesis that dispersed gastric cells possess two binding sites that have different affinities for the various H_2 antagonists. Occupation of the high-affinity binding sites is sufficient to inhibit the changes in cellular cyclic AMP.

INTRODUCTION

The existence of specific antagonists capable of interacting with the H_2 receptor has been well established (1, 2). However, the exact structural requirements of the H_2 receptor in gastric mucosa is still unknown despite the large body of research on a variety of *in vivo* and *in vitro* preparations (2–12). The aim of these investigations was to identify a safe and specific drug for the treatment of duodenal ulcer disease and some related conditions. The best H_2 antagonist in use today, cimetidine, has been proven also to have undesirable actions, including endocrine and central nervous system side effects (3, 13–15). In attempts to eliminate these undesirable side effects a number of new H_2 antagonists have been developed (9–12). Recently, it was suggested that the ethylthiomethyl ring-side chain of various guanidine derivatives is the critical structural feature of H_2 receptor antagonists (9). It is clear that the imidazol ring is not an essential component of an H_2 antagonist, since several studies have shown that the H_2 receptor could interact with non-imidazole compounds. Some of these compounds, such as tiotidine (thiazole derivative) (10) and ranitidine (furan derivative) (11), resemble the structure of cimetidine, whereas others (e.g., tricyclic antidepressants, lysergic acid diethylamide) do not (16, 17). Undoubtedly, more studies in the area of H_2 antagonism are necessary, particularly since reports were published showing discrepancies between the affinities for the antagonists obtained on histamine-stimulated acid secretion and those obtained on smooth muscle contraction (18) or on [^{3}H] cimetidine binding to gastric mucosal membranes (19).

We reported that in dispersed mucosal cells isolated from guinea pig stomach, histamine increased both cyclic AMP (20) and [^{14}C]aminopyrine uptake (21) and that cimetidine inhibited competitively the action of histamine on both processes by preventing the interaction of histamine with its membrane receptors (22). In the same preparation, various histamine analogues also increased cyclic AMP and [^{14}C]aminopyrine uptake and inhibited the binding of [^{3}H]histamine (22, 23). However, the action of some histamine analogues on [^{3}H]histamine binding was different from their action on the biological response (23). Thus, the biological response as reflected by measurement of acid formation or cellular cyclic AMP may not always reflect the event on the membrane

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receptors. Subsequently we proposed that gastric cells possess two binding sites for histamine and that occupation of one site is sufficient for the biological response.

To test further the possibility that guinea pig gastric mucosal cells possess more than one binding site and to explore the structural requirement of these sites, we examined and compared the effects of seven H_2 antagonists on binding of [^{3}H]histamine and on histamine-stimulated increase in cellular cyclic AMP.

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]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 from Sigma Chemical Company (St. Louis, Mo.); analytical precoated silica gel TLC[1] plates were obtained from Whatman, Inc. (Clifton, N. J.); precoated thin-layer cellulose on plastic sheet plates were from Polygram Cel, Macherey-Nagel and Company (Duren, West Germany). Cimetidine, metiamide, and compounds SKF 92629 and SKF 92408 were gifts from Dr. C. R. Ganellin, Smith Kline & French Laboratories (Welwyn Garden City, Herts., England). Tiotidine (ICI 125,211) was a gift from Dr. T. O. Yellin, ICI Americas Inc. (Wilmington, Del.). Ranitidine was a gift from Dr. F. N. Eshelman, Glaxo Group Research Ltd. (Fort Lauderdale, Fla.). Compound BL 5641A (etintidine) was a gift from Dr. D. G. Colton, Bristol Laboratories (Syracuse, N. Y.). 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

Preparation of gastric cells. Dispersed gastric cells from guinea pig stomach were prepared as described previously (23, 24). Cyclic AMP content and binding of [^{3}H]histamine to gastric cells were determined as previously described (20, 22, 23).

TLC of ^{3}H-labeled compounds. Aliquot samples from various [^{3}H]histamine solutions (e.g., original stock solution, solutions at the end of incubation, and supernatants after dissociation) were placed as single spots on a silica gel plate (5 × 20 mm) with or without unlabeled histamine as standard. The compounds were separated in an ethanol/ammonium hydroxide (4:1, v/v) solvent system for 100 min. The chromatograms were air-dried and the spots corresponding to histamine (R_F = 0.68) were scraped from the plates and assayed for radioactivity. In other TLC analyses we used precoated cellulose plates of 0.1 mm and 5 × 20 cm in a butanol/ethanol/ammonium hydroxide (8:1:3, v/v) solvent system as described elsewhere (25). Aliquot ^{3}H-labeled samples (20 μl) were applied in a 3- to 5-mm wide band across the plate with authentic histamine as standard. The chromatograms were air-dried, and 5-mm wide sections were scraped from the plates into a glass vial for assay of radioactivity.

[1] The abbreviation used is: TLC, thin-layer chromatography.

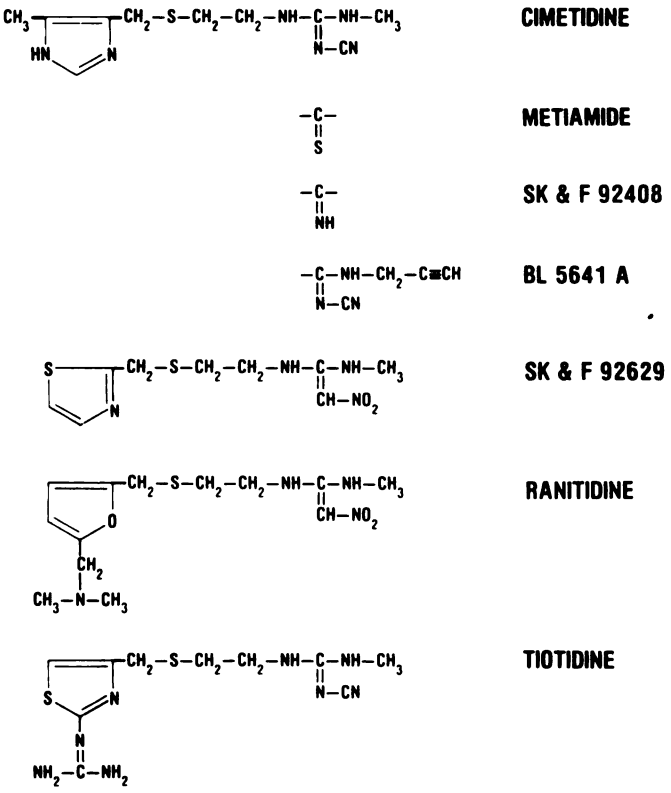

FIG. 1. *Chemical structure of H_2 antagonists*

RESULTS

Effect of histamine H_2 antagonists on cellular cyclic AMP. The H_2 antagonists used in the present studies were derivatives of 5-methyl 4-imidazole (cimetidine, metiamide, SKF 92408, and BL 5641A), derivatives of thiazole (SKF 92629 and tiotidine), and a derivative of furan (ranitidine). Their chemical structures are illustrated in Fig. 1. In dispersed mucosal cells from guinea pig stomach, histamine caused a 16-fold increase in cellular cyclic AMP with a half-maximal increase occurring at 20 ± 8 μM histamine (Fig. 2). The H_2 antagonists tested did not alter cellular cyclic AMP, but each antagonist inhibited the increase in cyclic AMP caused by histamine. At relatively low concentrations of histamine, each antagonist abolished the increase in cellular cyclic AMP and caused a parallel rightward shift in the dose-response curve for histamine. With sufficiently high concentrations of histamine, the inhibition caused by these agents

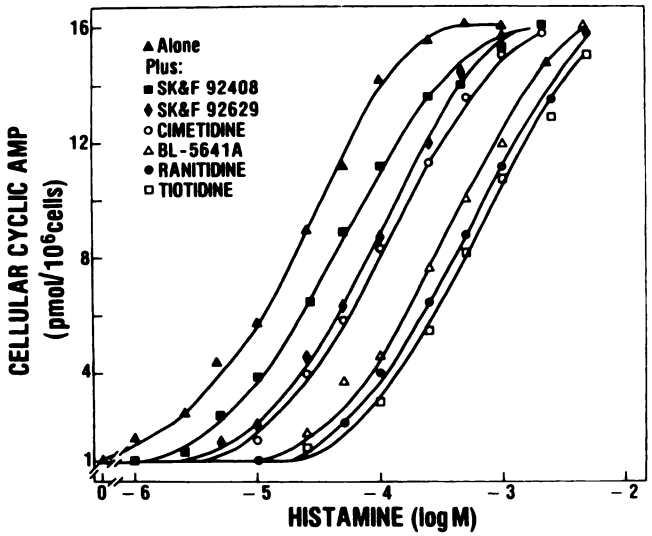

FIG. 2. *Effect of histamine and histamine H_2 receptor antagonists on cellular cyclic AMP in dispersed mucosal cells from guinea pig stomach*

Cells were incubated with varying concentrations of histamine with or without the indicated antagonists. All concentrations of antagonists were 3 μM (except tiotidine, 2 μM). Each point represents the mean of triplicate determinations; this experiment is representative of four others.

could be abolished. If the potency[2] of cimetidine is assigned a value of 100, the relative potencies of these antagonists in inhibiting the action of histamine on cellular cyclic AMP were as follows: tiotidine, 2000; ranitidine, 540; BL 5641A, 370; SKF 92629, 87; and SKF 92408, 26.

The action of the antagonists was further investigated by examining the ability of various concentrations of H_2 antagonist to inhibit the increase in cellular AMP caused by two different concentrations of histamine (Fig. 3). The increase in cellular cyclic AMP caused by 20 μM histamine was reduced by 50% with 2.1 μM SKF 92629 and was abolished by 100 μM SKF 92629 (Fig. 3A). With higher concentrations of histamine (100 μM), higher concentrations of SKF 92629 were required to cause inhibition comparable in magnitude to that seen with 20 μM histamine (Fig. 3A). A similar pattern of action was seen using ranitidine (Fig. 3B), BL 5641A (Fig. 3C), tiotidine (Fig. 3D), SKF 92408 (results not shown), and cimetidine (6); that is, increasing the concentration of histamine increased the concentration of antagonist required to produce 50% inhibition. However, sufficiently high concentrations of each antagonist were able to abolish the action of both concentrations of histamine.

Additional analyses were performed as described by Arunlakshana and Schild (26), using results obtained with histamine alone and with four different concentrations of each antagonist (Fig. 4). We found that increasing the concentration of the antagonist caused a parallel displacement of the histamine dose-response curve. The slope of the line relating log [$DR-1$] to log [antagonist] was not significantly different from unity (Fig. 4). These results are compatible with the antagonists tested acting as competitive inhibitors to the action of histamine on cellular cyclic AMP.

Effect of H_2 antagonists on [^{3}H]histamine binding. To explore the interaction between these antagonists and the receptors mediating the increase in cyclic AMP, we examined their ability to inhibit binding of [^{3}H]histamine to dispersed gastric mucosal cells. Each of the antagonists tested inhibited binding of [^{3}H]histamine, and sufficiently high concentrations of each antagonist abolished [^{3}H]histamine binding. However, the *relative* potencies with which these antagonists inhibited [^{3}H]histamine binding were different from those with which they inhibited the histamine-stimulated increase in cyclic AMP (Fig. 5). In inhibiting [^{3}H]histamine binding, the relative potencies of the antagonists were tiotidine > SKF 92408 > BL 5641A > cimetidine > metiamide > SKF 92629 > ranitidine. Compound SKF 92408 was among the most potent in inhibiting [^{3}H]histamine binding but the least potent on cyclic AMP, whereas ranitidine, which was the least potent in inhibiting [^{3}H]histamine binding, was very potent on cyclic AMP. Furthermore, the affinities of the receptors for the antagonists calculated from results on binding did not agree with the affinities for the antagonists calculated from cyclic AMP measurements. For example, ranitidine was 500-fold more potent in inhibiting cyclic AMP stimulation (K_i 0.15 μM) than in inhibiting [^{3}H]histamine binding (K_d 79 μM) (Fig. 5). These differences in the calculated parameters were also seen with tiotidine, SKF 92629, cimetidine, metiamide, and BL 5641A (Table 1). All of the antagonists tested were less potent in inhibiting [^{3}H]histamine binding than in inhibiting the action of histamine on cyclic AMP. The exception was compound SKF 92408, which had similar potencies for both processes (Table 1).

We considered the possibility that the antagonists were acting as *partial* rather than *fully* competitive antagonists in our system. The antagonists could interact with other sites in the vicinity of the receptors and by so doing alter the true affinity of the receptors for histamine. This might increase the concentration of antagonist required to compete with [^{3}H]histamine on binding to the receptors. If the antagonist alters the true affinity of the receptors for histamine, it must also alter the association or the dissociation between [^{3}H]histamine and the receptors. We explored this possibility by examining the effect of cimetidine on the dissociation of bound [^{3}H]histamine from gastric cells. Cells were incubated with [^{3}H]histamine for 60 min, washed to remove free radioactivity, and resuspended in fresh standard solution containing no radioactivity. We found that the loss of bound [^{3}H]histamine followed a first-order process with a dissociation rate coefficient of 0.04/min (Fig. 6). Adding 1 mM histamine or 1 mM cimetidine to the incubation solution did not change the rate of dissociation. Furthermore, analyzing the dissociated radioactivity after 40 min of incuba-

[2] "Potency" is calculated in terms of the concentration of antagonist required to occupy 50% of the receptors in the absence of histamine: the lower this concentration the higher the potency. K_i is the dissociation constant between the antagonists and the receptors from cyclic AMP measurements. K_d is the concentration of agent required to produce a half-maximal inhibition of tracer binding. All parameters were calculated as previously described (6, 23).

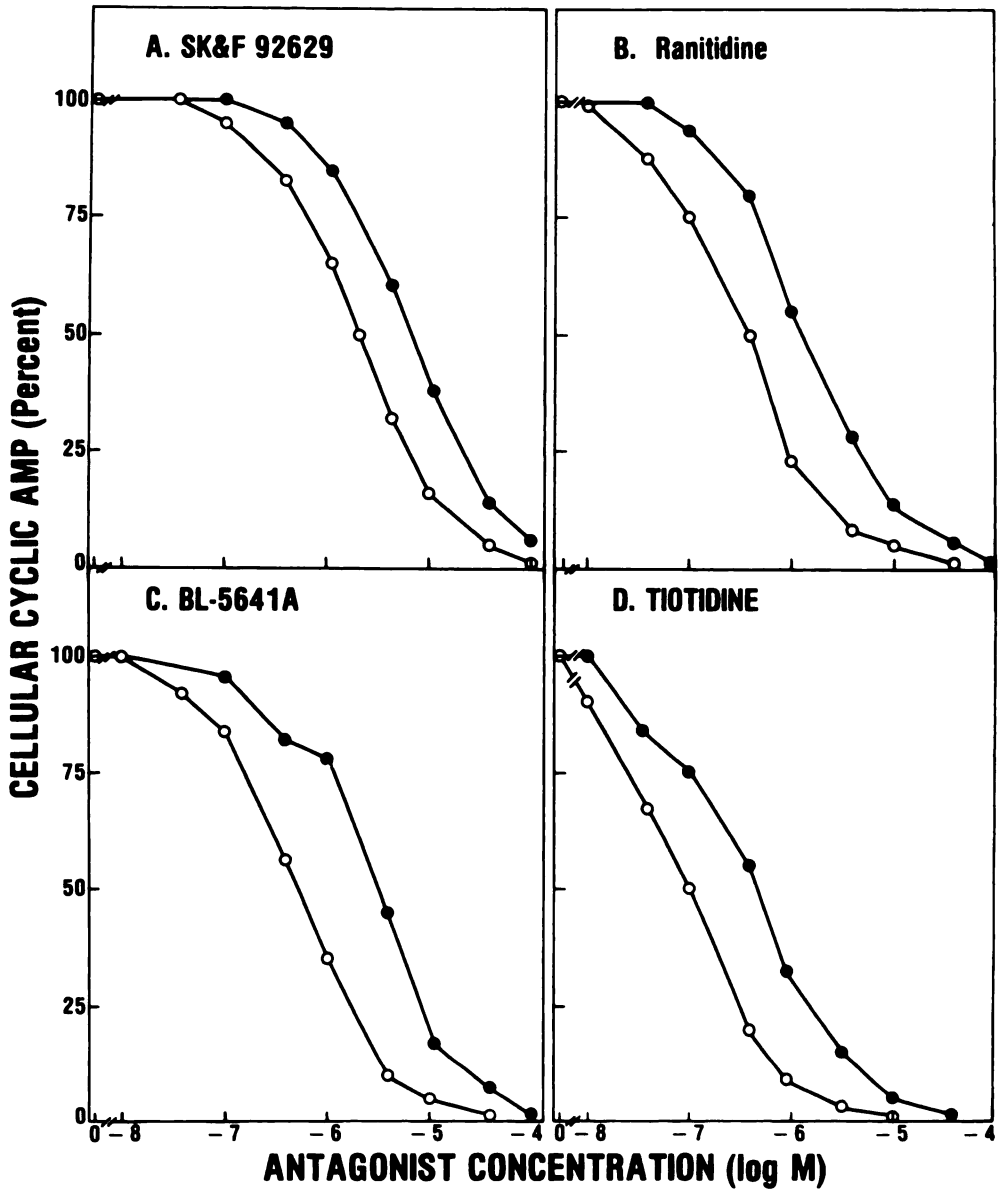

FIG. 3. *Effect of H_2 antagonists on histamine-stimulated increase in cyclic AMP in dispersed mucosal cells from guinea pig stomach*
Cells were incubated with varying concentrations of the indicated antagonists plus two different concentrations of histamine: ○, 20 μM histamine; ●, 100 μM histamine. Results are expressed as the percentage of the response obtained without the antagonists. Each point represents the mean of triplicate determinations; this experiment is representative of three others.

tion by TLC using two different systems revealed that 80%–90% migrated as [^{3}H]histamine and was chemically identical with authentic histamine (Fig. 7).

Relationship between inhibition of binding and cyclic AMP. Additional analyses were performed using the results presented in Figs. 2 and 5. The K_i values for the antagonists, calculated from results on cyclic AMP, were compared with the K_d values calculated from results on [^{3}H]histamine binding (Table 1). Plotting for each antagonist the log [K_d] on the Y axis as a function of the log [K_i] on the X axis could reveal whether the actions of these antagonists are mediated by a common receptor. A linear relationship between the two sets of values with a regression line approximating unity would suggest that inhibition of both [^{3}H]histamine binding and cyclic AMP stimulation are mediated by a common class of receptors and that these receptors have equal sensitivity for both processes. However, we found that there was no correlation between the two sets of values ($r = 0.09$), and the regression line describing these relations was not significantly different from zero (Fig. 8). When the two sets of values for ranitidine and compound SKF 92408 were omitted from the regression analysis, the obtained regression line was 0.328 ± 0.1 with a correlation coefficient of 0.88. The slope of this regression line was significantly different from both zero ($p < 0.05$) and unity ($p < 0.01$).

DISCUSSION

The present studies showed that all H_2 antagonists tested inhibited the histamine-induced increase in cellu-

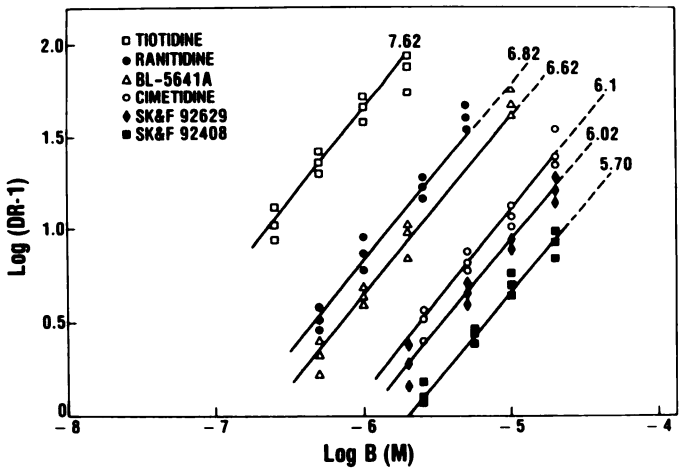

FIG. 4. *Schild plot representation of the effect of H_2 antagonists on histamine-stimulated increase in cellular cyclic AMP*

Cells were incubated as described in the legend to Fig. 2. In each experiment, performed on separate days, four concentrations of both histamine and each antagonist were used. Results are expressed according to the method of Arunlakshana and Schild (26). Each point represents triplicate determinations, and these results represent two experiments for each antagonist. The slope of the line for each antagonist was fitted by least squares and was not significantly different from unity, with 95% confidence limits of 0.85–1.07. The calculated pA_2 values (i.e., minus log [K_i]) are indicated at the top of the line for each antagonist.

lar cyclic AMP as well as the binding of [^{3}H]histamine to mucosal cells isolated from guinea pig stomach. The relative potencies with which these antagonists inhibited the increase in cyclic AMP were similar to their relative potencies on functions which are considered to be mediated by H_2 receptors (e.g., gastric acid secretion and contraction of atrial or uterine smooth muscles) (1, 3, 4, 10, 18, 27). Therefore, we concluded that the actions of these antagonists in our system reflect their interaction with histamine H_2 receptors. Since there was an agreement between binding of [^{3}H]histamine and histamine-induced increases in cellular cyclic AMP and [^{14}C]aminopyrine uptake (22), we are further suggesting that these antagonists interact with the H_2 receptors located on the parietal cells.

TABLE 1

Comparison of the K_d values obtained from binding analysis and the K_i values from cyclic AMP analysis of histamine H_2-receptor antagonists

All values are means ± 1 SD of the number of experiments given in parentheses.

Antagonist	Cyclic AMP, K_i[a]	Binding, K_d[b]
	μM	μM
Cimetidine	0.81 ± 0.20 (4)	12.0 ± 2.0 (4)
Metiamide	1.41 ± 0.41 (9)	16.0 ± 5.0 (3)
Ranitidine	0.15 ± 0.06 (6)	79.0 ± 10.0 (6)
Tiotidine	0.04 ± 0.02 (9)	5.4 ± 1.7 (5)
BL 5641A	0.22 ± 0.06 (5)	10.3 ± 1.9 (3)
SKF 92408	3.05 ± 1.02 (8)	5.9 ± 1.2 (3)
SKF 92629	0.93 ± 0.27 (8)	22.1 ± 9.2 (4)

[a] K_i values were calculated from the action of the antagonists on histamine-stimulated increase in cellular cyclic AMP (Figs. 2 and 3; ref. 6).

[b] K_d values were calculated from the computer-fitted data for inhibition of [^{3}H]histamine binding as described in the text.

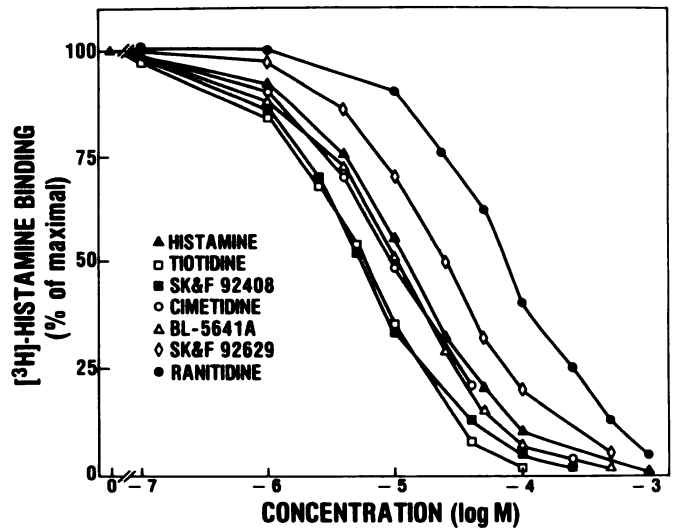

FIG. 5. *Inhibition of [^{3}H]histamine binding by histamine and histamine H_2 receptor antagonists in dispersed mucosal cells from guinea pig stomach*

Cells were incubated with varying concentrations of histamine or H_2 antagonist plus a tracer amount of [^{3}H]histamine (25 nM). Results are expressed as the percentage of the saturable portion of [^{3}H]histamine binding (i.e., 100%) obtained with the tracer alone (0.17 ± 0.01 pmole/10^6 cells). Each point represents the mean of triplicate determinations; this experiment is representative of three others.

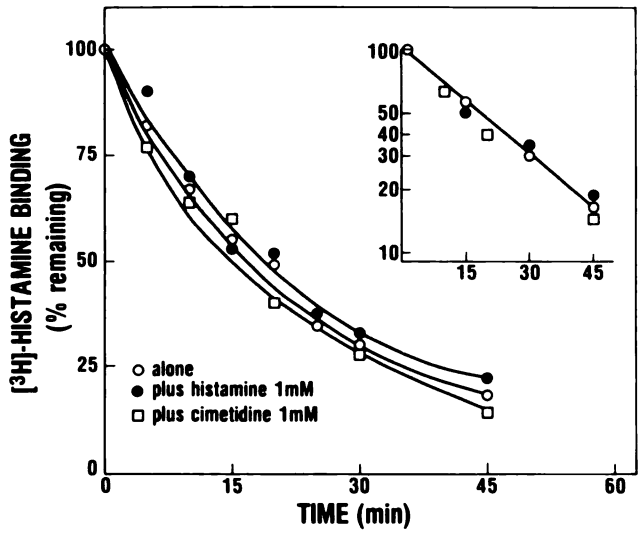

FIG. 6. *Effect of histamine and cimetidine on the dissociation of bound [^{3}H]histamine from dispersed gastric mucosal cells*

Cells were preincubated with [^{3}H]histamine (30 nM) for 60 min at 37°, washed to remove free radioactivity, and resuspended in fresh standard solution. The cells were incubated with or without 1 mM histamine or 1 mM cimetidine at 37°. Results are expressed as the percentage of the radioactivity present at the beginning of the incubation. *Inset,* same results expressed as the natural logarithm of the fraction of radioactivity remaining versus time in minutes. Each point represents the mean of triplicate determinations; this experiment is representative of four others.

The action of the antagonists on the functions measured (i.e., binding or cyclic AMP) was compatible with the antagonist competing with histamine for occupation of a single class of sites. The K_i value for each antagonist was the same whether it was calculated from experiments in which the concentration of histamine was varied in the presence of a constant concentration of the antagonist (Fig. 2) or from experiments in which the concentration of the antagonist was varied (Fig. 3). This K_i value was also close to the one obtained from Schild plot analyses (Fig. 4). Similarly, the dose response for each antagonist for inhibition of [^{3}H]histamine binding had a single component with no apparent evidence for more than one class of binding sites. However, when we compared the parameters for each antagonist on both processes, we found that there was a discrepancy between the two sets of values, suggesting that the two processes are not mediated by a common class of binding sites. The present observations resemble our previous findings on histamine receptor agonists in that the calculated parameters for the agonists obtained from binding studies were different from those obtained from cyclic AMP measurements (23). We found that with impromidine, dimaprit, or nordimaprit, the increase in cyclic AMP was not a linear function of their ability to inhibit [^{3}H]histamine binding. We proposed that gastric mucosal cells possess

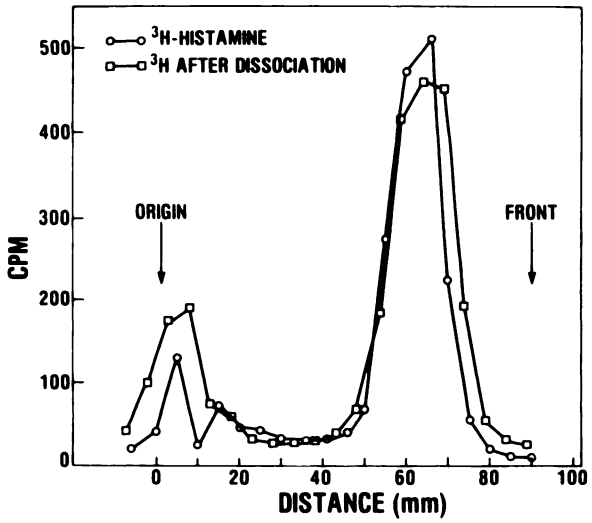

FIG. 7. *Thin-layer chromatography of ^{3}H-labeled compounds following dissociation from dispersed gastric mucosal cells*

Cells were treated as described in the legend to Fig. 6. After 40-min incubation at 37° (during which 75% of the radioactivity was dissociated), the supernatant was analyzed on thin-layer cellulose plates as described under Methods. Thin sections were scraped from the plates and assayed for radioactivity. Results are expressed as counts per minute per 5-mm wide section. A sample of the supernatant after dissociation was compared with the original stock solution of [^{3}H] histamine. In this system, chemical histamine has an R_F of 0.7, and the location of other authentic standards is described in ref. 25. In similar experiments, aliquot samples were separated on a silica gel TLC plate with ethanol/ammonium hydroxide (4:1, v/v) as the solvent system. We found that the spot corresponding to authentic histamine (R_F = 0.68) contained over 90% of the radioactivity with no significant difference between the samples taken from the various [^{3}H]histamine solutions (results not shown).

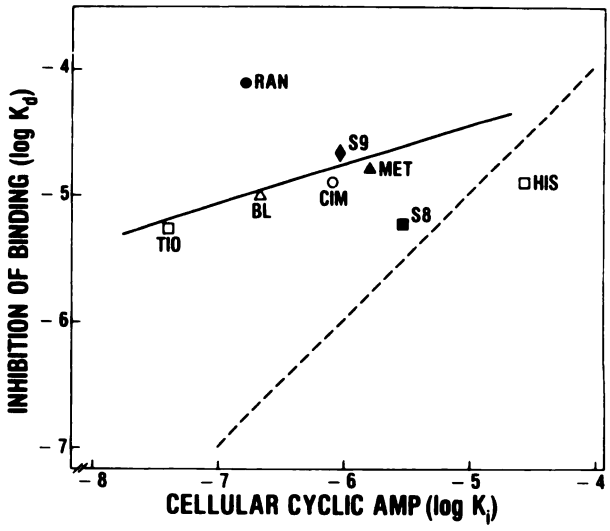

FIG. 8. *Comparison of the parameters that characterize effects of histamine H_2 antagonists on cyclic AMP and [^{3}H]histamine binding*

Values for K_i were taken from Figs. 2 and 3, and values for K_d were taken from Fig. 4. The value for histamine (*HIS*) represents the concentration of histamine required to cause a half-maximal increase in cellular cyclic AMP and was inserted for comparison. – – –, The unity line; *TIO*, tiotidine; *RAN*, ranitidine; *BL*, BL 5641A; *CIM*, cimetidine; *MET*, metiamide; *S9*, SKF 92629; *S8*, SKF 92408. The slope of the regression line (excluding the values for ranitidine and compound SKF 92408) was 0.328 ± 0.1 (r = 0.88), but when all values were included the regression line was not significantly different from zero (r = 0.09).

two binding sites for histamine and only a small fraction of these sites mediates the biological response (23).

Our present results agree with this hypothesis. Each antagonist binds to both binding sites, but only one site (site A) mediates the changes in cyclic AMP. The affinities of the two binding sites for the individual antagonist may not necessarily be identical. Therefore, the apparent affinity would depend on whether it was calculated from results on binding or from results on cyclic AMP. For example, the calculated affinity for tiotidine when measuring cyclic AMP reflected the affinity between one binding site (site A) and tiotidine because only binding to these sites mediates the changes in cyclic AMP (K_i = 0.04 μM). The calculated affinity when measuring inhibition of binding was determined by the concentrations of antagonist required to inhibit [^{3}H]histamine binding. The higher this concentration, the lower the affinity of the binding sites for the antagonist. Since with tiotidine higher concentrations were required for inhibiting binding than for inhibiting cyclic AMP formation, the calculated affinity from binding reflected the low affinity between a second binding site (site B) and tiotidine (K_d = 5.4 μM). Thus, gastric cells possess high- and low-affinity binding sites for tiotidine as well as for each of the antagonists tested (except SKF 92408), and the high-affinity binding sites mediate the changes in cellular cyclic AMP. Furthermore, the number of high-affinity sites for the antagonists constitutes less than 10% of the total binding sites. This latter proposition might be reflected by our inability to detect the high-affinity sites

by the binding studies. Compound SKF 92408, like histamine and its methylated derivatives (23), bound to both sites with a similar potency because the calculated parameters from each process (i.e., binding and cyclic AMP) were similar (Table 1).

Our present findings are similar to those obtained with [^{3}H]cimetidine. The inhibition of [^{3}H]cimetidine binding to membranes from guinea pig gastric mucosa by histamine-like compounds did not correlate with their potencies as reported from pharmacological experiments (19). Finally, as others have reported, non-imidazole compounds such as tricyclic antidepressants (16), lysergic acid diethylamide (17), and pyridyl, furan, and thiazole derivatives can interact with histamine H_2 receptors (5, 10, 11). Thus, the structural requirements for the H_2 receptor cover a wide range of compounds, and as we demonstrated in the present studies the events on the binding sites may not necessarily reflect the biological response. Obviously, further studies of this and other species will provide insight into the similarity between various animal species and contribute to the exact structural requirements needed to improve the interaction of future antagonists with the H_2 receptors.

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