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H_2 Histaminic Receptors in Rat Cerebral Cortex. 2. Inhibition of [^{3}H]Histamine by H_2 Antagonists†

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ABSTRACT: Sites labeled by [^{3}H]histamine in homogenates of rat cerebral cortex reveal a pharmacological specificity typical of H_2 receptors. Fourteen H_2 antagonists inhibit the specific binding of the radioligand to the same level; Hill coefficients are near or equal to one for five compounds and markedly lower for nine. The binding patterns of individual antagonists (A) are well described by the empirical expression $Y = F_1K_1/(K_1 + [A]) + F_2K_2/(K_2 + [A])$, in which F_1 and F_2 sum to 1; F_2 is 0 for those drugs that reveal a Hill coefficient of 1. Concentrations of A that reduce specific binding by 50% (IC_{50}) correlate well ($r = 0.991$; $P < 0.00001$) and show good numerical agreement with potencies reported for inhibition of the response to histamine in H_2-mediated systems. The correlation is poorer when IC_{50} is replaced by either K_1 ($r = 0.973$) or K_2 ($r = 0.921$) for those antagonists that reveal both; the antihistaminic activity of the drug thus appears not to be associated preferentially with one or other class of sites. Since F_2 varies from 0.16 to 0.60 among those antagonists that discern heterogeneity, the antagonist appears to determine the distribution of sites between the two classes. Moreover, a correlation among antagonists between values of K_1 and K_2 ($r = 0.975$; $P = 0.00001$) suggests that the apparent heterogeneity reflects different conformers within an otherwise homogeneous population. H_2 antagonists appear to be noncompetitive with respect to each other and to the radioligand: one antagonist has relatively little effect on the values of K_1, K_2, and F_2 revealed by another; also, estimates of K_1 and K_2 are independent of the concentration of [^{3}H]histamine between 1.3 and 10 nM, although the radioligand exhibits an apparent dissociation constant of 3.9 nM [Steinberg, G. H., Eppel, J. G., Kandel, M., Kandel, S. I., & Wells, J. W. (1985) *Biochemistry* (preceding paper in this issue)].

Evidence presented in the preceding report indicates that sites labeled by [^{3}H]histamine in homogenates of rat cerebral cortex respond to guanylyl nucleotides and magnesium in a manner that implies the presence of a nucleotide-specific G/F protein, and establishes the identity of the sites as neurohumoral receptors (Steinberg et al., 1985a). The binding of histamine is shown to be a complex process that cannot be understood in terms of conventional models for the interaction between neurohumoral agents and their receptors but appears to involve an interconversion of receptors between at least two states of affinity. Neurohumoral modulators acting via the G/F protein, and histamine itself, all appear to regulate the

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Table I: Mean Parametric Values for the Inhibition of [^{3}H]Histamine by H_2 Antagonists[a]

no.	H_2 antagonist	pharmacological potency[b] (pA_2)	eq 3 n_{H1}	eq 4					
				$-\log K_1$	$-\log K_2$	F_2	$-\log IC_{50}$[c]	$\log (K_2/K_1)$	ΔB_{min}[d] (%)
1	burimamide (2)	5.11[i]	1.006 ± 0.004	4.945 ± 0.007		[e]	[f]		−0.3, 1.1
2	SK&F 91581 (2)	3.90[j]	1.005 ± 0.011	4.008 ± 0.027		[e]	[f]		0.0, 3.0
3	imipramine (2)	6.39[k]	0.981 ± 0.002	6.007 ± 0.002		[e]	[f]		−0.1, 0.0
4	mepyramine (2)	5.30[l]	0.976 ± 0.019	4.760 ± 0.018		[e]	[f]		−3.9, −1.0
5	tiotidine (2)	7.43[m]	0.971 ± 0.008	7.253 ± 0.004		[e]	[f]		−0.5, 0.4
	tiotidine[g] (2)		0.958 ± 0.009	7.222 ± 0.005	6.641 ± 0.095	0.137 ± 0.011	7.150 ± 0.000	0.581	−0.3, −0.1
6	etintidine (2)	6.70[n]	0.795 ± 0.004	6.695 ± 0.005	5.219 ± 0.004	0.157 ± 0.001	6.497 ± 0.055	1.476	−0.2, 0.2
7	SK&F 92374 (2)	3.75[o]	0.937 ± 0.005	3.989 ± 0.004	3.023 ± 0.008	0.278 ± 0.002	3.754 ± 0.003	0.966	−1.4, −1.2
8	SK&F 92540 (2)	6.70[o]	0.784 ± 0.013	6.692 ± 0.014	5.571 ± 0.064	0.286 ± 0.023	6.423 ± 0.030	1.121	0.2, 0.3
9	cimetidine (2)	6.55[p]	0.593 ± 0.002	6.826 ± 0.017	5.131 ± 0.051	0.328 ± 0.011	6.417 ± 0.001	1.695	0.2, 0.3
	cimetidine[h] (2)		0.589 ± 0.002	6.841 ± 0.005	5.157 ± 0.001	0.346 ± 0.001	6.396 ± 0.006	1.684	−3.6, 0.6
10	SK&F 92408 (3)	4.80[q]	0.777 ± 0.040	4.996 ± 0.099	3.930 ± 0.147	0.422 ± 0.093	4.573 ± 0.021	1.066	−1.3, 0.3
11	SK&F 93479 (3)	7.78[r]	0.566 ± 0.011	8.228 ± 0.085	6.448 ± 0.039	0.498 ± 0.037	7.332 ± 0.060	1.780	−0.3, 0.0
12	SK&F 92422 (3)	5.15[o]	0.834 ± 0.008	5.508 ± 0.034	4.665 ± 0.048	0.577 ± 0.041	5.013 ± 0.011	0.843	0.0, 0.9
13	ranitidine (2)	7.20[p]	0.779 ± 0.025	7.728 ± 0.041	6.657 ± 0.067	0.598 ± 0.055	7.065 ± 0.013	1.071	−1.0, −0.3
14	SK&F 92629 (3)	5.96[s]	0.753 ± 0.003	6.206 ± 0.012	5.080 ± 0.013	0.598 ± 0.009	5.502 ± 0.006	1.126	−1.3, 0.9

[a] Values listed in the table reflect best fits of eq 3 ($n = 1$) and 4 ($n = 1$ or 2) to the data from individual experiments; the number of experiments is indicated in parentheses. The concentration of [^{3}H]histamine was between 1.23 and 1.44 nM unless indicated otherwise. [b] For all antagonists but imipramine, potencies are for inhibition of the chronotropic effect of histamine in guinea pig right atrium; for imipramine, potency is for inhibition of the histamine-stimulated accumulation of cAMP in homogenates of guinea pig hippocampus. [c] For each experrment, IC_{50} was calculated from the values of K_1, K_2, and F_2 derived from fitting eq 4 and indicates the concentration of antagonist that inhibits the specific binding of [^{3}H]histamine by 50%. The value listed in the table is the mean from replicate experiments. [d] ΔB_{min} was calculated according to the expression $\Delta B_{min} = 100(B_{min} - B'_{min})/(B_{max} - B_{min})$, where B_{max} and B_{min} are taken from the best fit of eq 4; B'_{min} indicates binding in the presence of 1.0 mM unlabeled histamine and was measured in each experiment. The two values indicate the range of ΔB_{min} for replicate experiments. [e] F_2 was fixed at zero. [f] IC_{50} and K_1 are identical. [g] The concentration of [^{3}H]histamine was 10.1 and 9.98 nM. [h] The concentration of [^{3}H]histamine was 10.1 nM in both experiments. [i] Black et al. (1972). [j] Ganellin (1980). [k] Green & Maayani (1977). [l] Trendelenburg (1960). [m] Gajtkowski et al. (1983). [n] Cavanagh et al. (1980). [o] C. R. Ganellin, Smith Kline and French Research Ltd., personal communication. [p] Daly et al. (1981). [q] Durant et al. (1978). [r] Blakemore et al. (1981). [s] Smith et al. (1980).

distribution of receptors between the two states; moreover, the binding of histamine shows evidence of being a cooperative process. The present report demonstrates that H_2 antagonists inhibit the specific binding of [^{3}H]histamine with a pharmacological specificity that identifies the labeled sites as H_2 receptors. Various aspects of the inhibition suggest, however, that the antagonists act in a noncompetitive manner.

Materials and Methods

Histaminic Drugs and Other Chemicals. [^{3}H]Histamine was obtained from Amersham Corp. (40–54 Ci/mmol) and New England Nuclear (32.2 Ci/mmol). Unlabeled histamine and imipramine were obtained from Sigma. Ranitidine was kindly donated by Glaxo Canada Ltd., Toronto, tiotidine by ICI Americas Inc., Wilmington, DE, and etintidine by Ortho Pharmaceutical Corp., Raritan, NJ. All other H_2 antagonists and dimaprit were the generous gift of Smith Kline and French Research Ltd., Welwyn Garden City, U.K. Details regarding other chemicals are provided in the preceding paper (Steinberg et al., 1985a).

Preparation of Tissue and Binding Assays. Homogenates of cerebral cortex were prepared from male Wistar rats and assayed for the binding of [^{3}H]histamine as described in the preceding paper (Steinberg et al., 1985a). The reaction mixture contained 100 mM tris(hydroxymethyl)aminomethane (Tris), 10 mM $MgCl_2$, and 1.0 mM ethylenediaminetetraacetic acid (EDTA) adjusted to pH 7.48 with sulfuric acid. All experiments involved a single concentration of the radioligand and a range of concentrations of an unlabeled drug. In each case, binding also was measured in the presence of 1.0 mM unlabeled histamine in order to compare the maximal inhibition achieved by histamine and by the drug under investigation.

Analysis of Data and Statistical Procedures. Data were analyzed according to the equations and procedures described in the preceding paper (Steinberg et al., 1985a). For each experiment, the point measured in the absence of unlabeled drug was included in all analyses; the point measured in the presence of 1.0 mM unlabeled histamine was omitted but is presented in each figure for the purpose of comparison.

Results

Five H_2 antagonists inhibit the specific binding of 1.23–1.44 nM [^{3}H]histamine with Hill coefficients indistinguishable from 1 (compounds **1–5**, Table I). The correlation coefficient of neighboring residuals is either negative or not significant ($P_1 > 0.1$) with n_{H1} fixed at 1 and, in 7 out of 10 experiments, the decrease in the variance of residuals is negligible with optimal values of n_{H1} ($P_2 > 0.05$). The data thus are well described assuming a single class of sites (eq 4, $n = 1$), as illustrated with burimamide and imipramine in Figure 1; the addition of a second class is without significant effect on the variance of residuals ($P_3 > 0.1$) in 8 out of 10 experiments. Estimates of log K_1 are listed in Table I. Nine antagonists reveal Hill coefficients significantly less than 1 (compounds **6–14**, Table I). In 20 out of 22 experiments, neighboring residuals are highly correlated ($P_1 < 0.0007$) when n_{H1} is fixed at one; in all experiments, the variance of residuals is reduced substantially ($P_2 < 0.00002$) with optimal values of n_{H1}. The data are well described assuming two classes of sites (eq 4, $n = 2$), as illustrated with etintidine, SK&F 92422, and SK&F 93479 in Figure 1; in all experiments, the correlation of neighboring residuals is poor ($P > 0.1$) or negative, and the variance of residuals is reduced substantially ($P_3 < 0.0002$) from that obtained assuming one class of sites. All antagonists achieve a level of maximal inhibition virtually identical with that achieved by unlabeled histamine. For 34 of the 36 experiments summarized in Table I, total binding measured in the presence of 1.0 mM histamine differs from the asymptotic value of eq 4 (B_{min}) by an amount that represents less than 1.5% of inhibitable binding ($B_{max} - B_{min}$). All antagonists tested thus appear to preclude access of the radioligand to the same

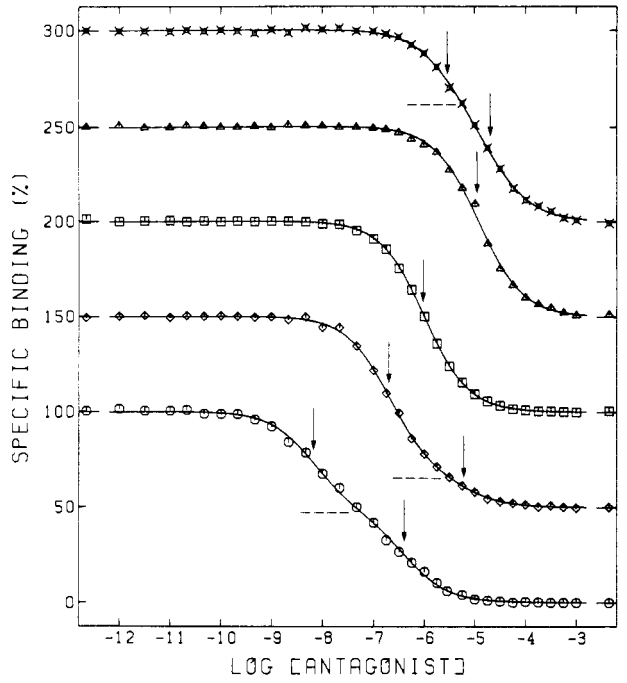

FIGURE 1: Inhibition of [^{3}H]histamine by H_2 antagonists at low concentrations of the radioligand. Total binding was measured following incubation of the suspension with [^{3}H]histamine (1.27–1.38 nM) and with the antagonist at the concentrations shown on the abscissa. The lines represent best fits of eq 4 (n = 1 or 2) to the experimental data; parametric values obtained by regression are as follows: for SK&F 93479 (○), $\log K_1 = -8.18 \pm 0.05$, $\log K_2 = -6.39 \pm 0.04$, and $F_2 = 0.47 \pm 0.02$; for etintidine (◇), $\log K_1 = -6.69 \pm 0.02$, $\log K_2 = -5.22 \pm 0.10$, and $F_2 = 0.16 \pm 0.02$; for imipramine (□), $\log K_1 = -6.01 \pm 0.00$; for burimamide (△), $\log K_1 = -4.94 \pm 0.01$; for SK&F 92422 (×), $\log K_1 = -5.54 \pm 0.11$, $\log K_2 = -4.68 \pm 0.07$, and $F_2 = 0.61 \pm 0.09$. Values plotted on the ordinate are normalized at 100% and 0% to the asymptotic values of eq 4 (B_{max} and B_{min}) obtained from the fitting procedure; successive curves are offset by 50% for clarity. Points at the lower and upper limits of the abscissa indicate binding in the absence of antagonist and in the presence of 1.0 mM unlabeled histamine, respectively; the latter was omitted from the regression. The arrows indicate $\log K_S$; F_2 is illustrated where appropriate by the dashed lines.

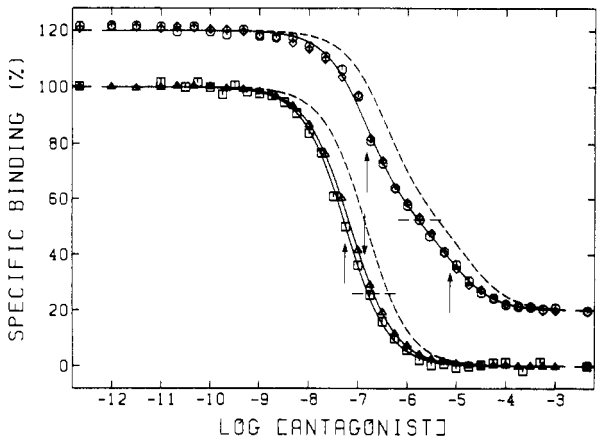

FIGURE 2: Inhibition of [^{3}H]histamine by tiotidine and cimetidine at two concentrations of the radioligand. Total binding was measured following incubation of the suspension with [^{3}H]histamine at either 1.36 (□, ○) or 10.1 nM (△, ◇) and with tiotidine (□, △) or cimetidine (○, ◇) at the concentrations shown on the abscissa. The solid lines represent the best fits of eq 4 to the experimental data. Cimetidine reveals two classes of sites at both concentrations of the radioligand, and the two sets of data were fitted simultaneously with common values for K_1, K_2, and F_2. Tiotidine reveals one and two classes of sites at 1.36 and 10.1 nM [^{3}H]histamine, respectively, and the two sets of data were fitted with a common value for K_1; F_2 was set to zero for the data at 1.36 nM [^{3}H]histamine. Parametric values obtained by regression are as follows: for cimetidine (○, ◇), $\log K_1 = -6.82 \pm 0.02$, $\log K_2 = -5.10 \pm 0.05$, and $F_2 = 0.33 \pm 0.01$; for tiotidine (□, △), $\log K_1 = -7.25 \pm 0.01$, $\log K_2 = -6.86 \pm 0.21$, and $F_2 = 0.26 \pm 0.08$. The two classes of sites observed with tiotidine at 10.1 nM [^{3}H]histamine are not well resolved; parametric values obtained from simultaneous analysis therefore differ somewhat from the mean values obtained from independent analyses and listed in Table I. The dashed lines are calculated according to eq 5 (n = 1 for tiotidine or 2 for cimetidine), and illustrate the binding pattern expected for each antagonist at 10.1 nM [^{3}H]histamine if all binding is fully competitive and the dissociation constant of the radioligand is 3.9 nM ($K_{P1} = K_{P2}$); values of K_{A1}, K_{A2}, and F_2 were obtained from the best fit of eq 5 ($K_{P1} = K_{P2} = 3.9$ nM) to the data acquired at 1.36 nM [^{3}H]histamine. The data for cimetidine are offset by 20% for clarity. The arrows and horizontal dashed lines indicate parametric values obtained with eq 4 as described above. Further details are given in the legend to Figure 1.

population of sites, in spite of the fact that the nonspecific fraction constitutes from 31% to 39% of total binding at about 1.4 nM [^{3}H]histamine. Parametric values obtained from eq 4 are highly reproducible from day to day and from batch to batch of tissue. The excellent agreement is illustrated by the results of simultaneous analysis in which duplicate or triplicate sets of data are fitted assuming common values of K_1, K_2, and F_2: the increase in the variance of residuals over that obtained assuming separate values for each replicate is negligible ($P_4 > 0.1$) with 10 compounds and small ($0.07 > P_4 > 0.03$) with 4.

An increase in the concentration of [^{3}H]histamine from 1.36 to 10.1 nM is without effect on the binding pattern of cimetidine, with no change in either the mean Hill coefficient or the mean parametric values derived from eq 4 (Table I). The agreement is illustrated in Figure 2, where data acquired at both concentrations of the radioligand have been fitted simultaneously (eq 4) assuming common values of K_1, K_2, and F_2 for the two experiments; there is no increase in the variance of residuals ($P = 0.62$) over that obtained with separate values for each set of data. A similar comparison can be made by using the four sets of data summarized in Table I. Common values of K_1, K_2, and F_2 fail to increase the variance of residuals over that obtained with two sets of parameters, one for each pair of curves ($P = 0.59$), or over that obtained with four sets of parameters ($P = 0.92$). There is no loss of reproducibility at the higher concentration of radioligand ($P_4 = 0.97$), in spite of the fact that the nonspecific fraction of total binding has increased from about 35% to about 49%.

In contrast to cimetidine, the binding pattern revealed by tiotidine becomes more complex at higher concentrations of the radioligand (Figure 2). At 1.35 nM [^{3}H]histamine, the Hill coefficient of 0.971 is indistinguishable from 1, and the data are well described assuming a single class of sites. At 10 nM [^{3}H]histamine, however, the mean Hill coefficient decreases to 0.958, and individual values are significantly less than one for both experiments quoted in Table I ($P_1 < 0.0035$; $P_2 < 0.00001$); differences in the variance of residuals indicate that the fit of eq 4 is substantially better with two classes of sites than with one ($P_3 < 0.00001$). The mean value of $\log K_1$ at 1.35 nM [^{3}H]histamine (-7.253 ± 0.004) agrees closely with that at 10 nM (-7.222 ± 0.005), and the variance of residuals is not increased significantly ($P = 0.66$) when the four sets of data summarized in Table I are fitted assuming a single value of K_1 rather than separate values for each pair of curves. [^{3}H]Histamine-related changes in the binding of tiotidine thus appear not to reflect changes in K_1; rather, higher concentrations of [^{3}H]histamine appear to label sites of somewhat lower affinity for the antagonist ($\log K_2 = -6.641 \pm 0.095$) and not observed at lower concentrations of the radioligand (Figure 2). The total number of sites occupied by [^{3}H]histamine ($B_{max} - B_{min}$) increased from 17–20 pmol/g

Table II: Comparison of F_2 among H_2 Antagonists Revealing Two Classes of Sites[a]

no.		SK&F 92629	ranitidine	SK&F 92422	SK&F 93479	SK&F 92408	cimetidine[b]	SK&F 92540	SK&F 92374
6	etintidine	<0.00001	0.00001	<0.00001	<0.00001	<0.00001	<0.00001	0.0014	0.0021
7	SK&F 92374	<0.00001	0.076	<0.00001	0.0097	0.19	0.0036	0.13	
8	SK&F 92540	<0.00001	0.020	<0.00001	<0.00001	0.0015	0.37		
9	cimetidine[b]	<0.00001	<0.00001	<0.00001	<0.00001	0.054			
10	SK&F 92408	0.0046	0.31	0.13	0.17				
11	SK&F 93479	0.0053	0.74	0.029					
12	SK&F 92422	<0.00001	0.00073						
13	ranitidine	0.12							

[a] Values listed in the table were determined as described in the text and indicate the levels of significance (P) for differences in F_2 (eq 4) between pairs of antagonists. [b] Only those experiments at 1.36 nM [^{3}H]histamine are included in the comparison.

of protein at 1.35 nM to 70–96 pmol/g of protein at 10 nM. Since F_2 for tiotidine is only 0.14 ± 0.01 at the higher concentration, only about 18% of the increase in the number of sites occupied by the radioligand is attributable to sites of lower affinity for the antagonist. Tiotidine and cimetidine differ in that the relative numbers of sites exhibiting one or other affinity is sensitive to the concentration of [^{3}H]histamine for one antagonist but not for the other. Since the concentrations of radioligand were the same for both antagonists, the difference is at variance with the notion of two classes of noninterconverting sites.

For both tiotidine and cimetidine, the apparent affinity is unchanged between 1.35 and 10 nM [^{3}H]histamine. The higher concentration of [^{3}H]histamine is 22-fold less than its apparent dissociation constant at the sites of lower affinity described by Steinberg et al. (1985a) (K_{P2} = 221 nM), but the range encompasses its apparent dissociation constant at the sites of higher affinity (K_{P1} = 3.9 nM). Were all sites of higher affinity for the radioligand, as suggested by isotopic dilution below 1.5 nM [^{3}H]histamine (Steinberg et al., 1985a), competitive effects would lead to a 2.6-fold increase in K_1 and K_2 between 1.35 and 10 nM [^{3}H]histamine. Such a reduction in the apparent affinity of an antagonist is clearly incompatible with the experimental data, as illustrated by the dashed lines in Figure 2. A lower limit on the value of K_P for [^{3}H]histamine can be obtained by simultaneous analysis of the data acquired at both concentrations of the radioligand. The four sets of data obtained with cimetidine at 1.36 and 10.1 nM [^{3}H]histamine are well described in terms of eq 5 (n = 2) assuming a dissociation constant for [^{3}H]histamine of 1 μM ($K_{P1} = K_{P2}$) and common values of K_{A1}, K_{A2}, and F_2. Successive analyses indicate that the variance of residuals is independent of the value of K_P between 1 and about 0.08 μM ($P > 0.1$). The variance is increased, however, when the value of K_P is decreased further to 0.063 (P = 0.029), 0.050 (P = 0.0035), 0.040 (P = 0.00016), and 0.032 μM ($P < 0.00001$). Equation 5 is therefore consistent with the data for cimetidine provided that the dissociation constant of the radioligand exceeds approximately 0.07 μM. It follows that the observed independence of K_1 and K_2 would be consistent with competitive inhibition were all sites of lower affinity (K_P = 221 nM) for the radioligand. This is at odds, however, with the results of isotopic dilution not only below 1.5 nM [^{3}H]histamine but also at about 10.5 nM [^{3}H]histamine where only 62% of the labeled sites appear to be of lower affinity (Steinberg et al., 1985a). It thus appears inappropriate to describe the inhibitory behavior of tiotidine and cimetidine in terms of a strictly competitive model; rather, the antagonists seem to act, at least in part, via a noncompetitive effect on the affinity of the radioligand.

It is of interest to compare values of F_2 among the nine antagonists that reveal two classes of sites at low concentrations of [^{3}H]histamine. Mean values range from 0.157 with etintidine to 0.598 with ranitidine and SK&F 92629 (Table I). The affinities are not widely separated, however, with differences between K_1 and K_2 ranging from 60-fold with SK&F 93479 to only 7-fold with SK&F 92422. Six antagonists show differences of less than about 14-fold. Differences in F_2 between pairs of antagonists therefore were tested for significance by simultaneous analysis of multiple sets of data. Equation 4 was fitted to the data from replicate experiments with two drugs assuming a common value of F_2 for both but separate values of K_1 and K_2. The variance of residuals then was compared with that obtained assuming separate values of all three parameters, and the results are listed in Table II. In the comparison between SK&F 92408 and SK&F 92629, for example, six sets of data are involved. A total of 17 parameters is required for the first analysis and 18 for the second. Both analyses require six values of B_{max} and B_{min}, one for each experiment, and two values of K_1 and K_2, one for each antagonist. The first analysis requires one value of F_2, while two values are required for the second, one for each antagonist. Of the 36 possible combinations among the drugs listed in Table II, the difference in F_2 is highly significant for 15 ($P < 0.00001$) and insignificant ($P > 0.10$) for only 8. The number of sites ostensibly of one or other affinity thus depends upon the antagonist. Since the concentration of [^{3}H]histamine in any experiment never deviated by more than 9% from the mean value of 1.35 ± 0.01 nM, the data are incompatible with the notion of two classes of noninterconverting sites.

Since H_2 antagonists appear to inhibit [^{3}H]histamine in a noncompetitive manner, combinations of two unlabeled drugs were tested to determine whether or not they appear competitive among themselves. The results presented in Figure 3 illustrate the binding patterns of cimetidine in the presence of the H_2 antagonists tiotidine and SK&F 93479 and the H_2 agonist dimaprit, at concentrations that reduce specific binding of the radioligand by 50% (Figure 3, insets). All three drugs cause a small increase in the Hill coefficient for cimetidine (Table III); in each case, however, the value remains significantly below 1 ($P_1 < 0.00001$; $P_2 < 0.00001$), and two classes of sites are required for eq 4 adequately to describe the data ($P_3 < 0.00001$). Values of F_2 are unchanged from that found in the absence of a second inhibitor ($P_5 > 0.1$, Table III), and the steepening of the curve thus reflects changes in the values of K_1 and K_2.

The experimental data presented in Figure 3 are shown together with the binding patterns expected if all drugs compete with each other and with the radioligand. Putative dissociation constants (K_A) for tiotidine, SK&F 93479, and cimetidine were obtained by fitting eq 5 (n = 1 or 2) to data acquired with each compound alone at concentrations of [^{3}H]histamine below 1.5 nM. Since two affinities have been observed for histamine (Steinberg et al., 1985a), all possible combinations were examined. With tiotidine, only a single class of sites is revealed by the data, but the value of K_{A1} could

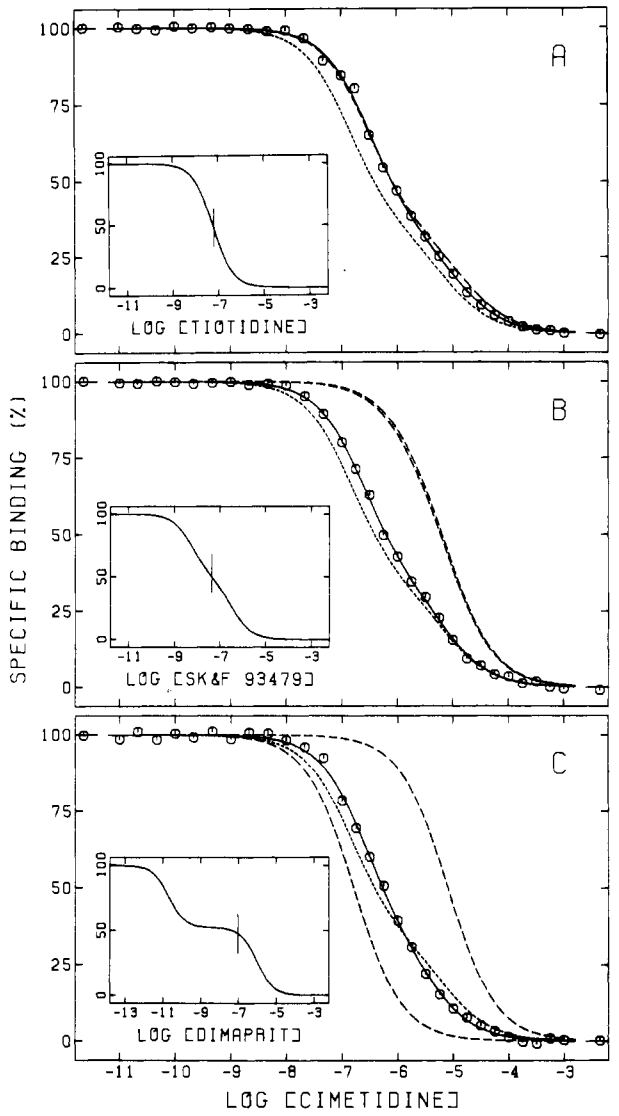

FIGURE 3: Effect of tiotidine (A), SK&F 93479 (B), and dimaprit (C) on the inhibition of [^{3}H]histamine by cimetidine. Total binding was measured following incubation of the suspension with 1.32–1.37 nM [^{3}H]histamine, cimetidine at the concentrations shown on the abscissa, and one of 0.063 μM tiotidine (A), 0.045 μM SK&F 93479 (B), or 0.10 μM dimaprit (C). The solid lines represent best fits of eq 4 (n = 2) to the experimental data; parametric values obtained by regression are listed in Table III. The dotted line in each frame illustrates the binding pattern of cimetidine in the absence of a second, unlabeled ligand and was calculated according to eq 4 (n = 2) with the parametric values listed in Table I (1.36 nM [^{3}H]histamine). The dashed lines were calculated as described in the text and illustrate the binding patterns expected if all ligands are competitive with respect to each other. The curve in each inset illustrates the binding of tiotidine, SK&F 93479, or dimaprit in the absence of cimetidine and is calculated according to eq 4 (n = 1 or 2) with the parametric values listed in Table I (<1.5 nM [^{3}H]histamine) of the present and following reports (Steinberg et al., 1985b); the vertical line indicates the concentration of the ligand that was present with cimetidine in the reaction mixture. In each experiment, the second unlabeled ligand reduced the specific binding of [^{3}H]histamine by about 50% in the absence of cimetidine. Further details are described in the legend to Figure 1.

be either 41 or 53 nM, depending upon whether the dissociation constant of [^{3}H]histamine is taken as 3.9 or 221 nM, respectively. With SK&F 93479 and cimetidine, two classes of sites give rise to four possibilities that in turn yield two estimates each for K_{A1} and K_{A2}; [^{3}H]histamine may bind to all sites with either higher or lower affinity or may exhibit a preference that is either the same as or opposite to that of antagonists. Evidence presented in the following paper (Steinberg et al., 1985a) indicates that all H_2 agonists inhibit the binding of 1.30–1.45 nM [^{3}H]histamine in a biphasic manner but that several, including dimaprit, exhibit anomalous properties that preclude analysis with eq 5. The dissociation constants for dimaprit therefore were taken as the values of K_1 (20.5 pM) and K_2 (1.00 μM) derived from eq 4 [Table I in Steinberg et al. (1985b)]. Estimates of the dissociation constants obtained as described above were used in an expression analogous to eq 5, but incorporating two unlabeled ligands rather than one, to calculate the binding patterns expected for cimetidine under the conditions listed in Table III. The computed curves are illustrated by the dashed lines in Figure 3. All possible combinations of affinity were tested within the following restrictions. First, the antagonists SK&F 93479 and cimetidine were assumed to show the same preference for the two classes of sites; that is, sites of higher affinity for one ligand are of higher affinity for the other. Second, the agonists dimaprit and [^{3}H]histamine were assumed to show the same preference with respect to each other, but not necessarily with respect to the antagonists. Third, the value of K_A for any ligand at either class of sites always was matched with the value of K_P, either 3.9 or 221 nM, that was used in its determination according to eq 5; the computed curves thus are consistent, at least with respect to apparent affinity, with the experimental data for either unlabeled ligand in the absence of the other. Fourth, the value of F_2 was taken as that found with eq 5 for cimetidine in the absence of a second, unlabeled ligand. The assignment of F_2 is arbitrary and disregards the differences found among different ligands. As noted above and in the following paper (Steinberg et al., 1985b), those differences are themselves at variance with the model underlying eq 5.

The experimental and computed binding patterns for cimetidine in the presence of 63 nM tiotidine are compared in Figure 3A. The various dissociation constants can be combined in four ways and yield the same result, which compares favorably with that obtained experimentally. Tiotidine thus alters the inhibitory behavior of cimetidine in a manner that resembles competitive inhibition. The overall change is small, however, and one cannot rule out the possibility that differences between competitive and noncompetitive effects fall within the limits of detection. While such differences are expected to be larger at higher concentrations of tiotidine, the ratio of specific to total binding is correspondingly lower. This problem is avoided with SK&F 93479, which reveals two classes of sites in contrast to the single class revealed by tiotidine. The 60-fold difference between K_1 and K_2 permits selection of a concentration ($\simeq K_1^{0.5}K_2^{0.5}$ M) that inhibits 88% of specific binding corresponding to F_1, but only 11% of that corresponding to F_2. The comparison in Figure 3B indicates that the experimental data differ markedly from the two curves that are predicted by the competitive model with all possible combinations of the various dissociation constants. It is noteworthy that the theoretical options include that in which the sites of both classes are of lower affinity for [^{3}H]histamine (K_P = 221 nM). While the inhibitory behavior of cimetidine would be unaffected by [^{3}H]histamine itself at experimentally practicable concentrations and thus appear noncompetitive with respect to the radioligand, it nevertheless is expected to undergo a major change in the presence of 45 nM SK&F 93479. The failure to observe such changes suggests that antagonists act in a noncompetitive manner with respect not only to [^{3}H]-histamine but also to other antagonists. The predicted effect of dimaprit is similar to that of SK&F 93479. At a concentration of 0.10 μM, this agonist inhibits over 99% of the

Table III: Effect of H_2-Specific Ligands on Parametric Values for the Inhibition of [^{3}H]Histamine by Cimetidine[a]

second unlabeled ligand	[[^{3}H]histamine]	eq 3 n_{H1}	eq 4 −log K_1 value	−log K_1 P_5[c]	−log K_2 value	−log K_2 P_5[c]	F_2 value	F_2 P_5[c]	ΔB_{min}[b] (%)
none[d]	1.36	0.59	6.83		5.13		0.33		
0.063 μM tiotidine	1.36	0.66 ± 0.02	6.45 ± 0.03	<0.00001	4.92 ± 0.07	0.0023	0.31 ± 0.02	0.29	0.7
0.045 μM SK&F 93479	1.37	0.67 ± 0.02	6.61 ± 0.03	<0.00001	5.13 ± 0.06	>0.99	0.34 ± 0.02	0.61	0.7
0.10 μM dimaprit	1.32	0.77 ± 0.02	6.58 ± 0.05	0.0013	5.39 ± 0.12	0.066	0.31 ± 0.05	0.70	0.8

[a] Values listed in the table reflect best fits of eq 3 ($n = 1$) or 4 ($n = 2$) to the data illustrated in Figure 3. [b] ΔB_{min} was calculated as described in footnote *d* to Table I, except that B_{max} was taken as total binding measured in the absence of both unlabeled ligands. [c] Level of significance for the difference in the variance of residuals between the best fit of eq 4 with five variable parameters (K_1, K_2, F_2, B_{max} and B_{min}), and that with K_1, K_2, or F_2 fixed at the mean value measured in the absence of a second, unlabeled ligand. [d] Parametric values are taken from Table I.

binding associated with F_1 and only 9.1% of that associated with F_2. The computed curves for cimetidine thus reveal a Hill coefficient of 1 and an apparent affinity of either 8.0 or 0.16 μM, depending upon the preference shown by cimetidine and dimaprit for sites of each class (Figure 3C). As in the example of SK&F 93479, differences between the experimental data and the computed curves suggest that dimaprit and cimetidine bind noncompetitively with respect to each other. A final comment relates to the small decrease observed in K_2 for cimetidine in the presence of dimaprit (Table III). This unusual change is opposite to that expected from competitive effects and may reflect the identity of the two ligands as an antagonist on one hand and an agonist on the other.

Discussion

The sites labeled by [^{3}H]histamine in homogenates of rat cerebral cortex reveal a pharmacological selectivity characteristic of H_2 receptors. All H_2 antagonists studied in the present investigation show good agreement between inhibitory potency with respect to the specific binding of [^{3}H]histamine and antihistaminic potency with respect to the H_2 chronotropic effect in guinea pig right atrium or, in the case of imipramine, the H_2-mediated accumulation of cyclic AMP. For the five antagonists that reveal a single class of sites, values of K_1 (eq 4) cover a 1760-fold range from 0.056 μM with tiotidine to 98 μM with SK&F 91581; potencies for the inhibition of binding and response correlate well ($r = 0.984$; $P = 0.0024$) and agree numerically to within a factor of 3.5 (Table I). It is noteworthy that the H_1 antagonist mepyramine, which is weakly antihistaminic in H_2 systems (Trendelenburg, 1960), inhibits the binding of [^{3}H]histamine with an apparent affinity 3500-fold weaker than its affinity for H_1 receptors in a similar preparation (Kandel et al., 1980).

For those antagonists that appear to reveal at least two classes of sites, a question arises as to which binding parameter is of pharmacological interest. On the assumption that the relevant parameter will be the same for all antagonists, −log K_1 and −log K_2 are compared with pA_2 in panels B and C of Figure 4, respectively. Both comparisons include values of −log K_1 for those antagonists that reveal a single class of sites. The correlation is highly significant for both parameters ($P < 0.00001$), but the correlation coefficient is higher with K_1 ($r = 0.973$; $t = 14.5$) than with K_2 ($r = 0.921$; $t = 8.19$); moreover, the value of −log K_1 is numerically closer to pA_2 for each of the nine compounds that discern heterogeneity. While this suggests that antihistaminic activity is more likely to reside in K_1 than in K_2, the highest correlation coefficient ($r = 0.991$; $t = 25.5$) is obtained by using the concentration of antagonist that reduces the specific binding of [^{3}H]histamine by 50% (log IC_{50}; Figure 4A). For seven of the nine compounds that discern heterogeneity, −log K_1 exceeds pA_2 by about 0.33 log unit, with individual ratios of potency varying from 1.6-fold to 3.4-fold; in contrast, pA_2 exceeds −log IC_{50} by about 0.25 log unit for eight compounds, with individual ratios varying from 1.4-fold to 2.9-fold. Values of −log K_1 and −log IC_{50} thus differ from pA_2 by about the same amount but in the opposite sense. The pattern shown by −log IC_{50} is the same as that found with four of the five compounds for which the Hill coefficient is 1 and the choice of parameter is unambiguous. This similarity is confirmed by the relatively small increase in the variance ($P = 0.033$) when the 14 compounds

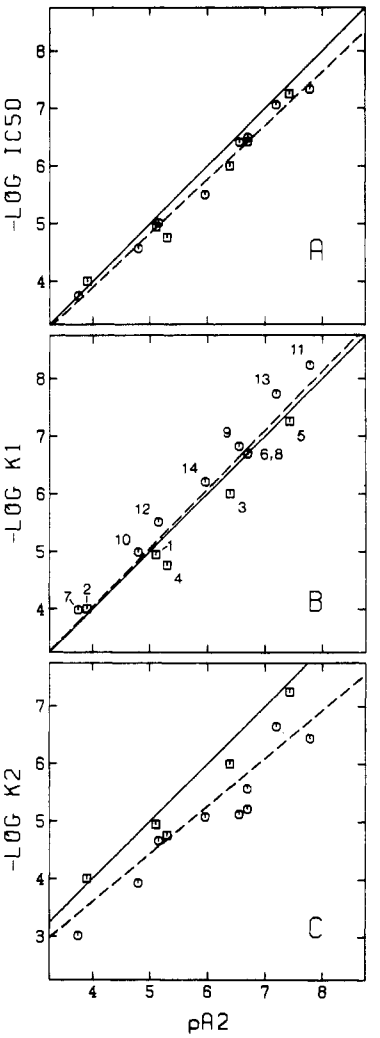

FIGURE 4: Comparison of binding parameters and H_2 pharmacological potency for H_2 antagonists. Antagonists are identified according to their numbers in Table I. H_2 pharmacological potency (pA_2) is compared in (A)–(C) with the mean, parametric values derived from best fits of eq 4 to the binding data; all values are listed in Table I. The symbols differentiate between those antagonists that reveal one (□) and two (○) classes of sites. Values of log K_1 and log K_2 are equal when only one class of sites is observed. Equivalence and correlation are illustrated by the solid and dashed lines, respectively, and all points contribute to the latter. Further details are given in the text.

are analyzed together, as illustrated in Figure 4A, rather than in two groups differentiated on the basis of the Hill coefficient (not shown). The increase in the variance is much larger for the analyses in panels B ($P = 0.00074$) and C ($P = 0.0043$) of Figure 4. These considerations suggest that IC_{50} is the parameter most appropriately compared with H_2 pharmacological potency. It follows that the antihistaminic activity of H_2 antagonists is directly proportional to the fraction of receptors rendered inaccessible to histamine and related only indirectly to whatever phenomenon determines the shape of the binding patterns.

The multisite model implicit in eq 4 and described more explicitly in eq 5 has been selected arbitrarily from among the several that predict Hill coefficients less than 1. Moreover, the upper limit of two on the degree of multiplicity revealed by any particular antagonist is based solely upon the observation that two classes of sites are sufficient for the model to describe the data. It thus is important to note that the two classes are not well resolved in the present data, owing primarily to the small differences (<61-fold) found among antagonists between K_1 and K_2. The nature of the binding patterns and the resolution of the data preclude tests for the possible involvement of more than two classes of sites, and arguments related to the applicability of this model must be qualified accordingly. Within the bounds of that qualification, however, the binding patterns of H_2 antagonists are clearly incompatible with the notion that all ligands bind competitively to a heterogeneous mixture of noninterconverting sites. First, the value of F_2 varies significantly from antagonist to antagonist. The variation seems to involve the same sites in each case, since the concentration of [^{3}H]histamine was virtually identical in all experiments being compared, and levels of maximal inhibition were indistinguishable for all drugs tested either alone or in combination. Second, the value of F_2 for one antagonist that discerns heterogeneity is not influenced by the addition of a second at a concentration sufficient to reduce binding corresponding to F_1 by 88% while having little effect on that corresponding to F_2. Third, values of K_1 and K_2 are not influenced by [^{3}H]histamine or other histaminic ligands in a competitive manner. The low Hill coefficients found among H_2 antagonists thus appear to arise from an antagonist-mediated interconversion of sites between two states of affinity. It is noteworthy in this regard that a binding pattern is defined by progressive increases in the concentration of an antagonist. The physical significance of F_2 is therefore uncertain, since that parameter seems unlikely to bear any simple relationship to the number of receptors in one or other state of affinity at any point in the curve. Although similar uncertainty is associated with K_1 and K_2, it is assumed here that those parameters reflect in some way the changes in free energy that accompany binding. The relationship may be highly indirect, however, and arguments involving K_1 and K_2 must be qualified accordingly.

Although implied by the data, the notion of multiple states of affinity is difficult to describe in terms of tentative models. If the system is at thermodynamic equilibrium, binding to a population of potentially identical and noninteracting sites is expected to be rectangular hyperbolic with respect to the concentration of free ligand irrespective of the number of conformational equilibria that may occur. Negatively cooperative systems describable by the Adair equation are ruled out by the differences in F_2, by the failure to observe changes in the binding of cimetidine at higher concentrations of the radioligand, and by the failure of SK&F 93479 to alter the binding of cimetidine. Previous authors have pointed out that low Hill coefficients can arise from a system in which a constituent of the membrane, presumably a macromolecule, interacts reversibly with the receptor to perturb the affinity of the ligand in an allosteric and reciprocal manner (De Haen, 1976; Boeynaems & Dumont, 1975; Jacobs & Cuatrecasas, 1976). A variant of this scheme applied to the β-adrenergic receptor (De Lean et al., 1980) is incompatible with the present data, however, as the values of log (K_2/K_1) and F_2 do not correlate in the expected manner (Wells, 1983). Moreover, the low Hill coefficients predicted by such models require that the receptor and the perturbing macromolecule be present in equal or nearly equal amounts and that they undergo rapid exchange on the time scale of a binding study. It follows that one antagonist would be expected to cause substantial changes in the binding pattern revealed by another, the changes arising from its influence on the equilibrium between the receptors and the perturbing macromolecules; this expectation is at variance with the minor effects of SK&F 93479 on the binding pattern of cimetidine. A major impediment to further analysis of the present data is the paradoxical behavior of histamine itself (Steinberg et al., 1985a). Until the binding of the radioligand can be described quantitatively, the mechanism of its inhibition is likely to remain unclear. Uncertainty over the inhibitory mechanism may account for some or all of the paradoxical effects associated with H_2 antagonists. One such example is the apparent failure of antagonists to compete even among themselves: the nature of their effect upon the binding of [^{3}H]histamine may obscure their effect upon each other.

Several observations support or at least concur with the notion that the heterogeneity revealed by some antagonists is induced in an otherwise homogeneous population of sites. First, F_2 varies from antagonist to antagonist; the appearance of heterogeneity thus is favored by some antagonists more than others. Second, five antagonists bind to an apparently homogeneous population of sites and thus appear to favor one state of affinity to the exclusion of the other. Third, all sites apparently participate in the blockade of histamine-evoked response, irrespective of the Hill coefficient of the antagonist; the phenomenon that determines the shape of the binding profile thus appears not to influence the intervention by the site in the histaminic response. Fourth, there is an excellent correlation ($r = 0.975$; $P = 0.00001$) between log K_1 and log K_2 among those antagonists that reveal heterogeneity of binding; a common pharmacological specificity for K_1 and K_2 implies that both contributions to the inhibition reflect binding of the antagonist at the same sites. The binding patterns of antagonists thus appear to reflect a single binding site that can exist in at least two states of affinity. The nature of the interconversion from one state to another remains obscure, however, except for the implication that it is controlled, at least in part, by the antagonist.

The complete absence of competitive effects between H_2 antagonists and [^{3}H]histamine raises the possibility that antagonists inhibit allosterically through binding at a site distinct from that occupied by the radioligand. Such a proposal is supported by the binding properties of receptors solubilized in 1% digitonin (Cybulsky et al., 1981; Wells et al., 1985; see also footnote 1). Upon dissolution of the membrane, the sites appear homogeneous with respect to the binding of histamine; the apparent dissociation constant in solution ($\simeq$5 nM) agrees well with K_{P1} in suspension (3.9 nM; Steinberg et al., 1985a), and sensitivity is retained to both magnesium and guanylyl imidodiphosphate (GMP-PNP). As described in the following

[1] D. L. Cybulsky, S. I. Kandel, and J. W. Wells, unpublished results.

paper (Steinberg et al., 1985b), there also is a retention of pharmacological specificity toward H_2 agonists. In contrast, there is a profound change in pharmacological specificity toward H_2 antagonists. Moreover, the inhibitory behavior of H_2 antagonists in the solubilized preparation is well described in terms of a competition between the antagonist and the radioligand for a single and uniform population of noninteracting sites. These changes are consistent with the notion that solubilization precludes an allosteric effect operative in suspension. If the inhibitory effect of H_2 antagonists in suspension is indeed allosteric, it presumably arises from a change in one or more of the rate constants for the interaction between the radioligand and the receptor. We have found, however, that the rate constant for dissociation of [^{3}H]histamine is the same irrespective of whether unlabeled histamine or ranitidine is used to block the forward reaction (Steinberg et al., 1985a). The similarity suggests that any allosteric or noncompetitive effects act to reduce the rate constant for association of the radioligand. The suggestion that H_2 antagonists inhibit noncompetitively raises the question of the identity of the antagonist-specific site and of the macromolecule on which it is located. It appears that the G/F protein is not involved, since the action of GMP-PNP on the binding of [^{3}H]histamine is retained in solution (Cybulsky et al., 1981). The existence of an antagonist-specific site in turn implies the existence of a natural ligand that presumably attenuates the action of histamine in vivo. Also, two classes of H_2 blockers are conceivable, differentiated on the basis of competitive or noncompetitive blockade with respect to histamine. If existing drugs are all of the noncompetitive type, the possibility may exist for a new and yet unrecognized class of H_2 antihistamines.

As pointed out in the preceding paper (Steinberg et al., 1985a), the sites ostensibly of higher affinity for [^{3}H]histamine (K_{PI} = 3.9 nM) are similar in several respects to those reported previously by Barbin et al. (1980). This similarity is reflected in the inhibitory behavior of H_2 agonists, as described in the following paper (Steinberg et al., 1985b), but not in that of H_2 antagonists. Barbin et al. (1980) found mepyramine, cimetidine, and metiamide all to reveal a Hill coefficient of 1. In contrast, the value of 0.59 listed for cimetidine in Table I is the second lowest found among antagonists in the present investigation. Although the affinities reported for mepyramine (K_i = 36.2 μM) and cimetidine (K_i = 14.0 μM) differ only by a factor of 2 from the present values of K_1 and K_2, respectively (Table I), the number of compounds is insufficient to suggest that the agreement is not fortuitous. An earlier report from the same laboratory implied that the Hill coefficients for H_2 antagonists were less than 1 (Palacios et al., 1978), but the inhibitory potency reported for cimetidine (IC_{50} = 63 μM) is 4.5-fold greater than the apparent affinity reported subsequently by Barbin et al. (1980). The several points of agreement between Barbin et al. (1980) and Steinberg et al. (1985a) suggest that the sites with nanomolar affinity for [^{3}H]histamine are the same in both reports. It therefore is surprising to find differences in the inhibitory properties of H_2 antagonists. Barbin et al. (1980) suggested that the sites labeled in their investigation might represent an inactive conformer of either H_1 or H_2 receptors. If H_2 antagonists are noncompetitive inhibitors of the radioligand, as suggested by the present data, the failure of Barbin et al. (1980) to observe binding of cimetidine comparable, for example, to that illustrated in Figure 2 may reflect an "uncoupling" of the antagonist-specific site from the H_2 receptor in their preparation. Gastric mucosal cells reportedly bind [^{3}H]histamine in a saturable manner, but with the very low affinity of 4–20 μM (Batzri, 1981); H_2 antagonists appear to inhibit the radioligand with Hill coefficients near or equal to 1 (Batzri et al., 1982), but the affinities show no relationship to those found in the present investigation.

Gajtkowski et al. (1983) recently have reported that homogenates of guinea pig cerebral cortex bind [^{3}H]tiotidine at an apparently uniform population of sites. Specific binding of the radioligand was inhibited by seven H_2 antagonists to yield estimates of affinity that correlate well with inhibitory potency in two H_2 systems: the histamine-stimulated accumulation of cyclic AMP in gastric mucosa and the histamine-induced chronotropic response in guinea pig right atrium. [^{3}H]Tiotidine thus appears to label the sites of pharmacological relevance for H_2 antagonists. For the five antagonists tested both by Gajtkowski et al. (1983) and in the present investigation, estimates of affinity at the [^{3}H]tiotidine-specific sites correlate well with the concentrations that reduce the specific binding of [^{3}H]histamine by 50% (r = 0.945; P = 0.015). Moreover, the capacity of 105 pmol/g of protein reported for [^{3}H]tiotidine is virtually identical with that found by Steinberg et al. (1985a) for the sites of higher affinity for [^{3}H]histamine. While these similarities suggest that [^{3}H]tiotidine and [^{3}H]histamine label the same sites, other observations suggest that the sites may be different. First, the Hill coefficient for cimetidine is 0.88 with [^{3}H]tiotidine (Gajtkowski et al., 1983) but only 0.59 with [^{3}H]histamine (Table I); the values for ranitidine are 0.94 and 0.78, respectively. Second, Gajtkowski et al. (1983) found that specific binding of [^{3}H]tiotidine could be demonstrated in cortical homogenates from guinea pig but not from rat, and that observation has been repeated recently in our own laboratory (unpublished results). Third, we demonstrate in the following paper (Steinberg et al., 1985b) that H_2 agonists inhibit the specific binding of [^{3}H]histamine in a unique and characteristic manner not reported with [^{3}H]tiotidine (Gajkowski et al., 1983). The suggestion that H_2 antagonists are noncompetitive inhibitors of the interaction between [^{3}H]histamine and the H_2 receptor raises the possibility that [^{3}H]tiotidine labels the antagonist-specific site rather than the H_2 receptor per se. Comparative studies with both radioligands in various tissues may clarify this matter.

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H_2 Histaminic Receptors in Rat Cerebral Cortex. 3. Inhibition of [^{3}H]Histamine by H_2 Agonists†

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ABSTRACT: The binding of [^{3}H]histamine to H_2 receptors in homogenates of rat cerebral cortex is inhibited by 11 H_2 agonists in a characteristic and unique manner. At low concentrations of the radioligand (<1.5 nM), the inhibitory profiles of individual agonists (A) are distinctly biphasic; specific binding is well described in most cases by the empirical expression $Y = F_1K_1/(K_1 + [A]) + F_2K_2/(K_2 + [A])$, in which F_1 and F_2 sum to 1. Maximal inhibition is the same for all agonists. Since values of F_2 vary from 0.42 to 0.90, the agonist appears to determine the equilibrium distribution of receptors between two states of affinity. Ratios of apparent affinity (K_2/K_1) vary from 204 to 3 090 000, and there is no correlation between values of K_1 and K_2. Compounds lacking H_2 activity, including structural analogues of histamine and dimaprit, reveal a Hill coefficient of 1 and inhibit the radioligand only weakly. For six agonists, values of K_2 agree and correlate well ($P = 0.00047$) with H_2 pharmacological potency (EC_{50}) in the guinea pig right atrium; for the others, K_2 is less than EC_{50} by 15–61-fold. Four observations suggest that the inhibition corresponding to F_1 is allosteric and cooperative: (a) the dissociation constant of the radioligand appears to vary in the presence of an unlabeled agonist, (b) absolute levels of binding corresponding to F_1, as defined by dimaprit, decrease at higher concentrations of [^{3}H]histamine, (c) F_1 for dimaprit is reduced from 0.48 to 0.32 by 2-methylhistamine ($F_1 = 0.27$) at a concentration of 20 nM ($\sim K_1^{0.5}K_2^{0.5}$ for 2-methylhistamine), but the increase in K_1 for dimprit is at least 100-fold less than expected from competitive effects, and (d) 1 equiv of some agonists appears to preclude access of [^{3}H]histamine to more than 1 equiv of receptors, with no evidence that an appreciable fraction of the unlabeled drug is bound. Noncompetitive effects also may account in part for the inhibition corresponding to F_2.

The sites labeled by [^{3}H]histamine in homogenates of rat cerebral cortex reveal a sensitivity to guanylyl nucleotides and magnesium that establishes their identity as neurohumoral receptors linked to a nucleotide-specific G/F protein (Barbin et al., 1980; Steinberg et al., 1985a). Evidence presented in the preceding paper indicates that the specific binding of [^{3}H]histamine is inhibited by H_2 antagonists in a complex manner characterized by Hill coefficients between 0.57 and 1.01 (Steinberg et al., 1985b). Since 50% inhibition is achieved at concentrations that agree well with reported estimates of pA_2 for blockade of the chronotropic effect of histamine in guinea pig right atrium, the sites labeled by [^{3}H]histamine

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