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Studies on Histamine H_2 Receptors Coupled to Cardiac Adenylate Cyclase

Blockade by H_2 and H_1 Receptor Antagonists

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SUMMARY

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In particulate preparations from guinea pig ventricle, histamine caused a three to five-fold stimulation of basal adenylate cyclase activity. The H_2 antagonist, cimetidine, displaced the dose-response curve of histamine to the right in a parallel fashion suggesting competitive antagonism. Schild plots were linear with slopes near one, consistent with a competitive inhibition mechanism. The affinity of cimetidine was independent of whether histamine or the pure H_2 agonist dimaprit was used to activate the enzyme. A series of ten H_2 antagonists related to cimetidine were examined for inhibition of histamine-stimulated cyclase activity. An excellent correlation was found between the affinities of these compounds for the H_2 linked cyclase system and for physiological H_2 receptors. Although several H_1 antagonists also appeared to be competitive inhibitors of the histamine-activated cyclase, their affinities did not correlate with data for H_1 receptors in the guinea pig ileum. The affinities of the antagonists, both H_2 and H_1, on the cardiac adenylate cyclase were virtually identical to their affinities for a histamine-stimulated cyclase from brain. The results suggest that the histamine-sensitive adenylate cyclase in ventricular muscle quantitatively retains the properties of an H_2 receptor system as defined physiologically and that the histamine receptors in ventricle and brain are not distinguishable. This study provides further strong evidence that histamine's inotropic and chronotropic effects on the intact heart are mediated by cAMP through H_2 receptor activation.

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INTRODUCTION

Histamine causes an increase in the rate and amplitude of the contraction of an isolated perfused guinea pig heart and these effects are preceded by increases in cAMP[1] levels (1, 2). These effects of histamine are completely and specifically blocked by the H_2 receptor antagonist burimamide but are poorly, if at all, antagonized by classical H_1 antihistamines such as mepyramine or tripelennamine (2–6). Similar results have been reported for papillary muscle or right ventricular strips from the guinea pig heart (7, 8). These studies suggest that the positive inotropic effects of histamine on the guinea pig ventricle are mediated by H_2 receptors and involve an elevation of cAMP levels in the cardiac muscle cells.

The role of cAMP in the inotropic actions of hormones is still a debated topic, and dissociations between the inotropic effects of catecholamines and glucagon and alterations in cAMP levels have been noted (9, 10). Although no such dissociations have been reported for histamine, this may reflect the fact that less research has been carried out with this substance than with the other inotropic agents. A major impetus for the present study was the desire to provide additional data for or against a role for cAMP in H_2 receptor-mediated events. At the enzymatic level, there are two major approaches to this problem. The first is to attempt to correlate quantitatively the potencies of a large series of agonists and antagonists on adenylate cyclase with their potencies on physiologically defined H_2 receptors associated with inotropism. The second is to show, with different species and tissues, that wherever there is a physiologically demonstrable H_2 receptor, there is also a histamine-activated adenylate cyclase having the pharmacological characteristics of an H_2 receptor.

Several laboratories have described histamine stimulation of adenylate cyclase activity in broken cell preparations from guinea pig ventricle (4, 11–15). Using a limited series of agonists and antagonists, we recently described a preliminary pharmacological characterization of the histamine-sensitive adenylate cyclase in guinea pig ventricle and concluded that this enzyme had the characteristics expected of an H_2 receptor system (14). In a more recent study (16), we extended these observations to a much larger series of agonists, we re-evaluated the structural requirements for H_2 receptor activation and how these results bear on our previously described theoretical model for receptor activation (17), and we examined the influence of guanylnucleotides on histamine activation of adenylate cyclase. In the present paper, we examined a large number of specific H_2 receptor antagonists and correlated their affinities on the histamine-activated adenylate cyclase from guinea pig ventricle with their affinities for physiological H_2 receptors. In addition, we have compared the properties of the histamine-activated cyclase from cardiac muscle and brain. Our studies on histamine sensitive adenylate cyclases in different species and tissues and their correlation with the physiological effects of histamine in these different tissues will be presented elsewhere.

EXPERIMENTAL PROCEDURES

Preparation of the cardiac particulate fraction. The ventricles from adult guinea pigs were homogenized in a medium containing 0.25 M sucrose, 5 mM Tris, 1 mM EGTA, pH 7.2, using a Polytron (3 times 5 seconds at medium speed) followed by a motor driven glass-teflon homogenizer (10 strokes at medium speed). Homogenates were filtered through four layers of cheesecloth and centrifuged for 20 minutes at 1000 *g*. The pellet was washed twice in the same medium by hand homogenization followed by re-centrifugation and the final pellet was suspended in the same medium at a protein concentration of 1–3 mg/ml. These crude particulate fractions, which contain most of the adenylate cyclase of the muscle, could be quick frozen in a dry ice-acetone bath and stored for months at −20° with no changes in basal activity or in the stimulation elicited by histamine.

Adenylate cyclase assay. All assays were performed in triplicate. All reagents except

[1] Abbreviations used: GppNHp, guanosine-5′-(β-γ-imino)triphosphate; cAMP, adenosine-3′-,5′-monophosphate; pA_2, negative log of the receptor-antagonist dissociation constant.

the labeled ATP were added to the assay tubes on ice (final volume 225 μl). They were then transferred to a 30° shaking incubator and preincubated for 5 minutes to allow the enzymatic activity to reach a steady state and to eliminate the influence of any lag periods in hormone activation. After the preincubation period, 25 μl of [α-^{32}P]ATP (1–2 μCi) were added and in most cases the reaction was allowed to proceed for 10 to 20 minutes when it was stopped by adding 100 μl of 1% sodium dodecyl sulfate. After addition of 650 μl of [^{3}H]cyclic AMP ([^{3}H]cAMP; 5000–10,000 cpm) to monitor recovery, the labeled cAMP was isolated with alumina and Dowex columns (18). The reaction was linear with protein concentration (19) in the range used and for at least 20 minutes after the addition of the [α-^{32}P]ATP. Unless otherwise noted, the assay medium contained (after addition of the labeled ATP) 90 mM Tris-HCl (pH 7.4), 1 mM ATP, 2 mM Mg^{2+}, 1 mM cAMP, 4 mM theophylline, 5 mM phosphocreatine, 8 units of creatine phosphokinase, 10^{-5} M GTP, 75 mM sucrose, 0.3 mM EGTA, and 75–225 μg membrane protein in a final volume of 250 μl.

Treatment of the data. Curve fitting techniques (14) were used to estimate the apparent ED_{50} values, maximum stimulation by agonists, and parallelism of the dose-response curves. In most cases, antagonist affinities were estimated using the dose ratio equation directly:

$$pA_2 = \log (DR - 1) - \log [\text{antagonist}]$$

but, in some instances, antagonism was analyzed in more detail by Schild plots (20). The dose ratio (DR) is the ratio of agonist concentrations needed to produce half-maximal responses in the presence and absence of antagonist. Simple competitive antagonism results in a straight line of unit slope when log (DR − 1) is plotted against log [antagonist]. The intercept with the abscissa (DR = 2) is the pA_2 value (negative log of the receptor-antagonist dissociation constant).

Materials. [α-^{32}P]ATP and GppNHp were obtained from ICN and [^{3}H]cAMP from New England Nuclear. Histamine, ATP, cAMP, theophylline, phosphocreatine, creatine phosphokinase, bovine serum albumin and GTP were from Sigma. Cimetidine and metiamide were provided by J. Paul (Smith Kline, Philadelphia). All other H_2 antagonists and dimaprit were the generous gifts of Dr. C. R. Ganellin (Smith Kline and French, England). Mepyramine maleate and cyproheptadine HCl were from Merck, tripelennamine HCl from Ciba, and diphenylpyraline from Smith Kline.

RESULTS

Kinetic studies. The time course for stimulation of guinea pig ventricle adenylate cyclase by histamine and its inhibition by the H_2 antagonist cimetidine is shown in Fig. 1. Activity was linear with time for at least 20 minutes following addition of the [α-^{32}P]ATP. Activity in the presence of histamine was independent of whether the hormone had been present from the beginning, that is, during the 5 minute preincubation, or whether it was added 12 minutes after the preincubation. In the latter case there was no evidence of a lag phase before reaching the fully activated state. The H_2 antagonist cimetidine was able to inhibit the stimulation induced by histamine, again with no significant lag phase, and the activity following addition of the antagonist was essentially the same as found when histamine and cimetidine were present from the beginning. The experiment in Fig. 1 was repeated exactly as shown except that the unlabeled Mg-ATP was not included in the preincubation but was added along with the [α-^{32}P]ATP at zero time. The results were virtually superimposable on the data of Fig. 1; again there was no discernible lag phase in the presence of histamine. We conclude that activation of the H_2 receptor and adenylate cyclase by histamine is rapid and readily reversible upon addition of antagonist.

Effects of H_2 antagonists. The inhibitory effects of the H_2 antagonist cimetidine on adenylate cyclase activity stimulated by histamine and the pure H_2 agonist dimaprit (21) are shown in Figs. 2 and 3, respectively. The data are from three experiments and are expressed as a percentage of the maximum stimulation obtained in each individual experiment. Expressing the data this

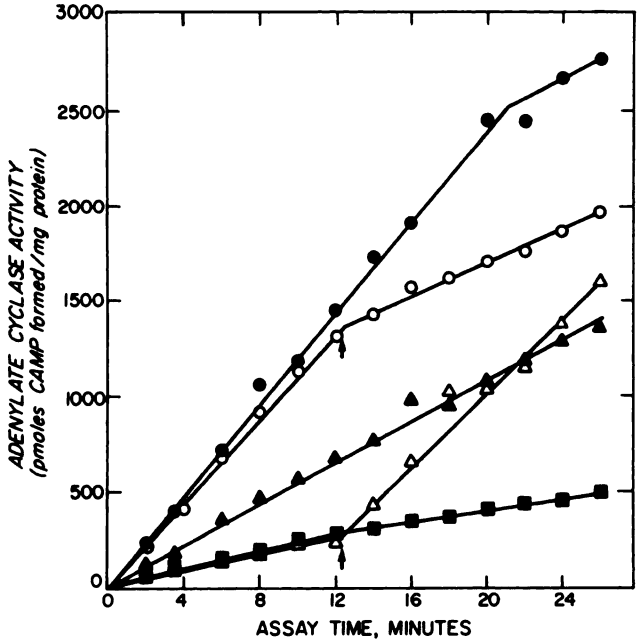

FIG. 1. *Time course for histamine activation of guinea pig ventricular adenylate cyclase and blockade by the H_2 antagonist cimetidine*

■, basal activity; △, basal activity with addition of 10^{-5} M histamine at the arrow; ▲, 10^{-5} M histamine + 50 μM cimetidine; ○, 10^{-5} M histamine with 50 μM cimetidine added at the arrow; ●, 10^{-5} M histamine. All assays were conducted in the presence of 10^{-5} M GTP.

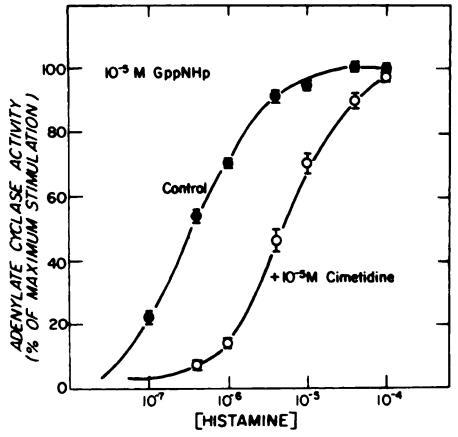

FIG. 2. *Dose response curves for histamine activation of guinea pig ventricular adenylate cyclase in the presence and absence of cimetidine*

●, absence of cimetidine; ○, presence of 10^{-5} M cimetidine. All assays were conducted in the presence of 10^{-5} M GppNHp. The data are expressed as a percentage of the maximal stimulation elicited by a saturating concentration of histamine and represent the means ± SEM for three experiments on different membrane preparations.

way is necessary due to the variation in the extent of activation obtained with histamine in different preparations.

In Fig. 2, the assay was conducted in the presence of 10^{-5} M GppNHp. As noted previously (16), this nucleotide causes a marked shift to the left in the dose response curve of the agonist as well as an increase in the relative magnitude of the maximal stimulation, as compared to dose response curves conducted in the presence of GTP. Cimetidine shifted the histamine dose response curve to the right in a parallel fashion suggesting competitive antagonism. From the dose ratios for the three individual experiments, a pA_2 value of 6.10 ± 0.06 was obtained. It should be noted that 10^{-5} M cimetidine consistently depressed basal activity by about 20% when the experiments were conducted in the presence of GppNHp. No depression of activity was observed in the presence of GTP. It is unclear whether this reflects a non-specific effect of cimetidine on GppNHp-activated cyclase or whether there might be small

quantities of histamine in the membrane preparations which can stimulate cyclase activity in the presence of GppNHp but not in the presence of GTP. This is possible since the cyclase is an order of magnitude more sensitive to histamine in the presence of GppNHp. Based on a histamine level of 5 μg/g of guinea pig heart (22) and assuming that all of this histamine remained bound to the membranes during isolation, one can calculate that assay concentrations as high as 10^{-7} M could be present. In order to generate Fig. 2, basal activity in the absence of cimetidine was used for the control dose response curves and basal activity in the presence of cimetidine was used for the experimental curves. If there are small quantities of histamine in the preparation, then this method of calculation would lead to small errors in the estimated pA_2 values. These errors are, however, well within the variation obtained with different preparations of membranes.

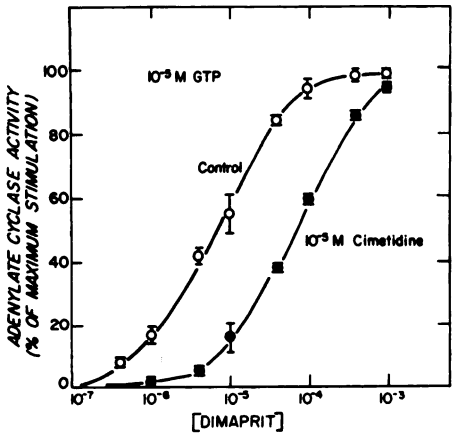

FIG. 3. *Dose response curves for dimaprit activation of guinea pig ventricular adenylate cyclase in the presence and absence of cimetidine*

○, absence of cimetidine; ●, presence of 10^{-5} M cimetidine. All assays were conducted in the presence of 10^{-5} M GTP. The data are expressed as a percentage of the maximal stimulation elicited by a saturating concentration of dimaprit and represent the means ± SEM for three experiments on different membrane preparations.

The results shown in Fig. 3 were obtained in the presence of GTP. Under these conditions, dimaprit acted as a partial agonist (16). Cimetidine caused a parallel shift in the dose response curve and a pA_2 value of 5.97 ± 0.06 was calculated for the three experiments.

All of our experiments with cimetidine are summarized in Table 1. The results suggest that the pA_2 value of cimetidine calculated from the Schild equation is independent of the concentration of antagonist, independent of the agonist used and whether it is a full or partial agonist, and independent of the type of guanylnucleo-

TABLE 1

Antagonism of histamine and dimaprit stimulation of cardiac adenylate cyclase by cimetidine

The table shows a compilation of data from eleven independent experiments performed on nine different membrane preparations. In each experiment, complete dose response curves to the agonist (either histamine or dimaprit) from 10^{-6} to 10^{-3} M were obtained in the absence or presence of cimetidine at the concentrations shown and with either 10^{-5} M GTP or 10^{-5} M GppNHp. The dose response curves were computer fit as described in the METHODS section, dose ratios were calculated from the ED_{50}'s of the fitted curves, and the pA_2 values calculated from the Schild equation. Using the histamine/GTP data only, a least squares fit of Log (mean dose ratio −1) vs. Log (Cimetidine) yielded a slope of 0.93, a correlation coefficient of 0.989 (values consistent with a simple competition mechanism), and an estimated pA_2 value of 6.21.

Agonist	[Cimetidine]	pA_2
	(M)	*(mean ± SEM)*
Histamine/GTP	3×10^{-6}	6.08, 6.28
	5×10^{-6}	6.09
	10^{-5}	6.18 ± 0.11 (4)
	3×10^{-5}	5.97
	10^{-4}	6.10
		group mean = 6.10 ± 0.04 (5)
Histamine/GppNHp	10^{-5}	6.10 ± 0.06 (3)
Dimaprit/GTP	10^{-5}	5.97 ± 0.06 (3)

tide present in the assay. The least squares fit of the histamine/GTP data (see footnote to Table 1) is consistent with a simple competition mechanism.

Correlation of antagonist affinities on the cyclase and physiological H_2 receptors. A large series of antagonists structurally related to cimetidine were examined for competitive inhibition of the histamine-activated adenylate cyclase. The affinities of the pure antagonists, in the form of pA_2 values, were calculated from dose ratios, the latter being obtained by computer fits (14) of the dose response curves in the absence and presence of antagonist. The concentrations of antagonist used were chosen to give about a ten-fold shift in the histamine dose response curve. Two of the compounds, imidazolylpropylguanidine and N^{α}-guanylhistamine, are also partial agonists. The affinities of these two compounds were determined by the standard pharma-

TABLE 2

Comparison of the affinities of antagonists for histamine-stimulated adenylate cyclase and for H_2 receptors in guinea pig atria and H_1 receptors in guinea pig ileum

The numbers shown in the second column represent the pA_2 values (negative log of the antagonist-receptor dissociation constant) for inhibition of histamine activated guinea pig ventricular adenylate cyclase expressed as means ± SEM for the number of experiments shown in parentheses. These values were calculated as described in the text. The data for H_2 receptors (chronotropic effect of histamine on guinea pig atria) and for H_1 receptors (histamine-induced contraction of the guinea pig ileum) were taken from the literature. All cyclase experiments were conducted in the presence of 10^{-5} M GTP.

Antagonist	Cardiac adenylate cyclase	Atrial H_2 receptors	Ileum H_1 receptors
H_2 Antagonists			
N-imidazolylpropyl-N′-methyl-thiourea (SKF91581)	3.23 ± 0.09 (3)	3.5[a]	
N^{α}-guanylhistamine (SKF71448)	3.87 ± 0.10 (3)	3.9[b]	3.8[b]
N-methyl-N′-[2-[(5-methylimidazol-4-yl)-methylthio]-ethyl] urea (SKF92166)	4.76 ± 0.05 (3)	4.66[a]	
imidazolylpropylguanidine (SKF91486)	4.86 ± 0.04 (3)	4.65[c]	
burimamide	4.96 ± 0.16 (3)	5.11[d]	3.5[d]
thiaburimamide (SKF92027)	5.38 ± 0.13 (4)	5.49[e]	
N-methyl-N′-[2-[5-methylimidazol-4-yl)-methylthio]-ethyl]guanidine (SKF92408)	5.56 ± 0.15 (4)	4.80[a]	
N″-carbamoyl-N-methyl-N′-[2-[5-methylimidazol-4-yl)-methylthio]-ethyl]guanidine (SKF92422)	5.76 ± 0.15 (3)	5.15[a]	
metiamide	5.97 ± 0.07 (4)	6.04[e]	
cimetidine	6.10 ± 0.05 (5)	6.10[f]	3.4[f]
H_1 Antagonists			
mepyramine	5.15 ± 0.04 (3)	[g]	9.4[h]
tripelennamine	5.36 ± 0.30 (3)		8.5[i]
diphenylpyraline	6.89 ± 0.05 (2)		9.8[j]
cyproheptadine	7.63 ± 0.02 (3)		8.3[k]

[a] Ref. 24.
[b] Ref. 25.
[c] Ref. 26.
[d] Ref. 27.
[e] Ref. 28.
[f] Ref. 29.
[g] Trendelenburg (ref. 30) reported an estimated pA_2 value of 5.3 on atrial rate but also noted that mepyramine caused a pronounced decelerating effect by itself and that it could not be stated with confidence that this compound was acting as a competitive antagonist of histamine.
[h] Ref. 20.
[i] Ref. 31.
[j] Personal communication from P. Ridley.
[k] Ref. 32.

cological approach commonly used to estimate partial agonist affinity (23). The affinities of the compounds for histamine-stimulated cardiac adenylate cyclase are shown in Table 2 along with a compilation of data from the literature on their affinities for H_2 and H_1 receptors in guinea pig atria and ileum, respectively. All the cyclase data shown in Table 2 were obtained in the presence of 10^{-5} M GTP in the assay medium. With the exception of SKF92422 and SKF92408, an excellent correlation was found between the cyclase results and the H_2 receptor data, the correlation coefficient being 0.99 and the slope very near unity (0.95). The reason for the deviation of these two compounds is not clear. Structurally, they are very close analogues of cimetidine:

(4-methylimidazole)- $CH_2SCH_2CH_2NH$- C(=X)-$NHCH_3$

	X
Cimetidine	N-CN
SKF92408	NH
SKF92422	N-$CONH_2$
SKF92166	O

Notice that both cimetidine and SKF92166 fit the correlation quite well. SKF92408 and 92422 were both more potent on the cardiac cyclase than on the atrial H_2 receptors.

Effects of H_1 antagonists. The limited data available on the potencies of the H_2 antagonists on H_1 receptors (see Table 2) clearly indicate that the structural factors involved in the binding to the H_1 receptor are quite different from the factors involved in the interaction with the histamine-activated adenylate cyclase. This is also demonstrated by our studies on several classical H_1 antagonists (Table 2). These compounds cause a parallel shift in the dose response curve of histamine on cardiac adenylate cyclase. However, their affinities for the cyclase system are totally unrelated to their affinities for the H_1 receptors in the ileum. These compounds have not been extensively studied on physiological H_2 receptors in high doses. At the doses that would be needed for H_2 receptor blockade, it seems likely that the well known non-specific depressions of contractility induced by these agents would obscure any specific antagonism of histamine.

Comparison of the histamine-activated adenylate cyclase in cardiac muscle and

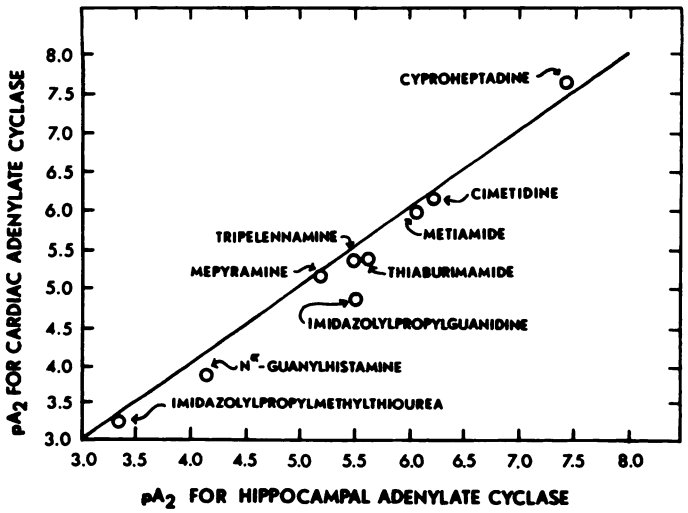

FIG. 4. *Comparison of the affinities of antagonists for inhibition of histamine-activated adenylate cyclase from guinea pig ventricle or hippocampus*

The affinities of the antagonists are expressed in the form of pA_2 values which are the negative log of the receptor-antagonist dissociation constant. The pA_2 values for the hippocampus are from our previous report (34). The pA_2 values for the cardiac enzyme are from Table 2. The line shown in the figure is the line of identity not a least squares fit.

brain. Histamine stimulates adenylate cyclase activity in membrane preparations from guinea pig hippocampus and cortex (33–38). Figure 4 shows the relationship between the antagonist affinities obtained on the guinea pig ventricle cyclase and the guinea pig hippocampal cyclase. Both H_2 and H_1 antagonists are included in the figure. It is clear that there are no substantial differences between the properties of the H_2 receptors in the two tissues, at least as far as antagonist affinities are concerned. There is a suggestion in the figure that the affinities of the antagonists for the brain receptor are slightly higher than for the the cardiac receptor. The differences are generally small and within the usual experimental variation seen from preparation to preparation. Nevertheless, with the exception of cyproheptadine, the differences are all in the same direction. The assay conditions for the two sets of data were identical and in many cases the same animals were used to provide the cardiac and brain membranes.

DISCUSSION

The studies presented in this paper demonstrate that histamine causes a rapid stimulation of cardiac adenylate cyclase activity with little or no discernible lag phase and that this activation of the enzyme can be readily reversed by addition of a specific H_2 antagonist, cimetidine. On the basis of the parallel shift to the right of the histamine dose response curve and the linear Schild plot with near unit slope, the blockade by cimetidine appears to be that expected of a simple competitive antagonist. The affinity of cimetidine for the cyclase-linked receptor is identical within experimental error to the affinity of this antagonist for physiologically defined H_2 receptors in the atria and uterus. The affinity of cimetidine calculated from the Schild equation (i.e., assuming unit slope) appears to be the same for both histamine (full agonist with mixed H_2 and H_1 activity) and dimaprit (partial agonist under our assay conditions; reported to be a pure H_2 agonist). Furthermore, despite the marked effect of the guanylnucleotide analogue GppNHp on the agonist dose response curve and the fact that activation of the cyclase in the presence of this nucleotide is essentially irreversible (14), the affinity of cimetidine in the presence of GppNHp is the same as in the presence of GTP.

We observed an excellent correlation, with two exceptions, between the affinities of a large series of H_2 antagonists for the histamine-activated cyclase and for physiological H_2 receptor data on the atrial chronotropic receptors for histamine. We have been forced to utilize a ventricular muscle cyclase system since we were unable to show significant stimulation by histamine of the adenylate cyclase in guinea pig right atria, presumably because of the small amount of sino-atrial nodal tissue in comparison to the total atrial tissue. Unfortunately, quantitative physiological data on the ventricle are practically non-existent in the literature. Two of the antagonists, SKF92408 and 92422, deviate significantly from the correlation and this is true even if the confidence limits on the published atrial data and our own cyclase data are taken into account. It should be emphasized, however, that our pA_2 values for these compounds are based only on calculations using the Schild equation, not from complete Schild plots. We have no evidence, therefore, other than the parallel displacement of agonist dose-response curves, that these compounds are acting strictly as competitive antagonists. It should also be noted that these two compounds, in contrast to cimetidine and SKF92166, are very basic guanidines. Possibly their greater activity in the cyclase assay than in the physiological assay is somehow related to this factor. On the other hand, the other guanidines studied, guanylhistamine and imidazolylpropylguanidine, fit the correlation quite well. It is possible that the deviation of SKF92408 and 92422 from the correlation obtained in this study reflects some subtle difference in the H_2 receptors mediating the positive chronotropic and positive inotropic effects of histamine. There is some evidence available at present to support the hypothesis of H_2 receptor subtypes. For example, the order of potency of the H_2 antagonists, burimamide and metiamide, on the guinea pig ventricle (39) was opposite to that for

the atria (28). However, the ventricular strip potencies were based on what appears to us to be extremely variable data. Nevertheless, such discrepancies are of concern and need to be resolved in future physiological studies of cardiac histamine receptors using a series of histamine H_2 antagonists with a reasonable spread in relative potency. More extensive comparisons have been made between the atrial and uterine H_2 receptors using twelve antagonists closely related to cimetidine (40). Although the overall correlation between the two sets of data was quite good, two of the compounds showed approximately a three-fold difference in their apparent affinities for the two receptors, being less active on the atrium than on the uterus. One of these compounds was SKF92408, the antagonist that deviated most from our own correlation study. The pA_2 values for this compound were 4.80 on the atrium, 5.26 on the uterus, and 5.56 ± 0.15 on the cyclase. Thus, use of the uterus data would improve our correlation considerably. The other compound that deviated somewhat from our correlation (SKF92422) had virtually the same potency on atria and uterus (40). The discrepancies noted above between affinities of antagonists for H_2 receptors in different tissues could imply receptor heterogeneity; however, there are many other explanations for such effects, including differences in tissue binding or metabolism, release of endogenous hormones, interaction with other receptors, etc. In general we feel that the available evidence supports the view that H_2 receptors are not distinguishable at present, at least with respect to inhibition by H_2 antagonists. This may include the H_2 receptors in the brain since we did not observe any quantitatively significant differences in the affinities of antagonists for the adenylate cyclase-linked histamine receptors in cardiac muscle and hippocampus (Fig. 4). In addition to the H_2 and H_1 antagonist data reported here, we have also examined on the cardiac cyclase several additional compounds previously studied on the hippocampal cyclase system, including D-LSD and brom-LSD (34, 36) and amitriptyline (36, 41). Our results on the cardiac enzyme (data not shown) are again in agreement with the published data on brain adenylate cyclase. However, as shown in Fig. 4, there does appear to be a systematic trend such that antagonists are slightly more active on the brain cyclase than on the cardiac muscle enzyme. A similar finding has been reported by Kanof and Greengard (15) who examined twelve antagonists, including six H_1 antagonists and four imidazole-N-methyl transferase inhibitors. In the latter study, the largest deviation represented about a six to sevenfold difference in affinity for the two receptors, whereas the largest difference seen in our study was about four-fold. The significance of these observations is uncertain. There may be some subtle differences between the H_2 receptors in brain and cardiac muscle. However, it is possible that in the cardiac membranes there is a small but significant binding of all of the antagonists to other sites, thus leading to an incorrect value for the free antagonist concentration and a systematic error in the calculation of the affinity or inhibition constants.

The present studies on a large series of H_2 antagonists, along with the data presented elsewhere (16) for a series of agonists, strongly support the hypothesis that the histamine-activated adenylate cyclase has the properties expected of an H_2 receptor system. It may be argued that, because the H_1 antagonists also appear to be competitive blockers of the histamine-stimulated cyclase and because these compounds are generally not effective on physiological H_2 receptors, the broken cell cyclase has lost its selectivity for the H_2 antagonists. This would seem to us to imply a fairly substantial modification of the nature of the receptor. It seems unlikely that the receptor would quantitatively retain its binding affinities for a large series of H_2 antagonists and yet lose its selectivity for these antagonists. Our view (14, 34) has been that the actions of the H_1 antagonists on the cyclase simply reflect the binding of rather high concentrations of these notoriously non-specific agents to the H_2 receptor. Because of the multiple effects of high concentrations of these agents, it may well be impossible to demonstrate specific antagonism of H_2 receptors in physiologically

intact systems. It is possible, therefore, that the H_2 receptor is relatively non-selective even in the intact tissue. This raises the interesting possibility that new H_2 antagonists may be derived from classes of compounds structurally distinct from the present series of H_2 blockers. This is particularly interesting to pursue since the most potent H_2 antagonist reported, cimetidine, has very low affinity in comparison to the blockers developed for other receptors. Cyproheptadine and several other tricyclic structures are in fact much more potent than cimetidine, at least in the cyclase assay. However, these compounds react with a variety of other receptors (41, 42 and references therein). What is clearly needed are compounds of high affinity as well as good selectivity for the H_2 receptor. A search for such structures is presently underway in our laboratory. We have recently found that several chemically diverse compounds commonly referred to as calcium antagonists are also H_2 antagonists in the cyclase assay. One of these, L-cis-diltiazem, was of fairly high affinity ($pA_2 = 6.94$) and displayed stereospecific blockade of the histamine-activated adenylate cyclase. Schild plots for both isomers were linear and the slopes were 1.07 and 1.05 for the L and D isomers, respectively. The L isomer was 32 times more active than the D isomer. Diltiazem had no influence on basal activity or on isoproterenol-stimulated activity. Furthermore, the more active stereoisomer on the cyclase was least active as a calcium antagonist suggesting that by structural modification of the parent compound it may be possible to obtain a selective H_2 antagonist having few, if any, non-specific side effects. Our studies on diltiazem and several other related compounds possessing apparent H_2 antagonist activity are described elsewhere (43).

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