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Pharmacological Properties of Histamine-Sensitive Adenylate Cyclase from Guinea Pig Cardiac Ventricular Muscle

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SUMMARY

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An adenylate cyclase that is stimulated by low concentrations of histamine (k_a, 6 μM) has been studied in cell-free preparations of guinea pig cardiac ventricular muscle. The characteristics of activation of this adenylate cyclase by histamine receptor agonists, and of inhibition of this enzyme by H_2-receptor antagonists (e.g., metiamide, cimetidine), indicate that this histamine-sensitive adenylate cyclase possesses pharmacological properties similar to those of an H_2-receptor as defined by physiological experiments in peripheral tissues. Thus, the activation of this adenylate cyclase by histamine may be an early step in the sequence of biochemical events through which histamine acting at H_2-receptors exerts its positive inotropic effects on cardiac ventricular muscle. The stimulation of adenylate cyclase activity by histamine was also competitively inhibited by several H_1-receptor antagonists (e.g., mepyramine, diphenhydramine, promethazine) and by several imidazole-N-methyl transferase (INMT) inhibitors (e.g., quinacrine, etoprine). The results indicate that many structurally diverse compounds known to interact with various histamine binding proteins (i.e., the H_2-receptor, as well as the H_1-receptor and the histamine catabolic enzyme INMT) are capable of interacting with H_2-receptors coupled to adenylate cyclase in heart. Furthermore, quantitative differences in the inhibition constants of H_2-receptor antagonists, H_1-receptor antagonists, and INMT inhibitors for the histamine-sensitive adenylate cyclases from guinea pig heart and brain suggest the existence of tissue heterogeneity in the pharmacological properties of H_2-receptors.

INTRODUCTION

Histamine exerts its physiological effects on various tissues by interacting with either of two types of receptors, designated H_1 and H_2 (1, 2). The contractile response of guinea pig ileum is one example of a response to histamine mediated by H_1-receptors (1), whereas the stimulation of the rate of contraction of guinea pig atria, the stimulation of acid secretion in gastric mucosa, and the inhibition of contractions of rat uterus are examples of responses to histamine mediated by H_2-receptors (2). The development of agonists (1–3) and antagonists (2, 4–6) selective for H_1- and H_2-receptors has facilitated the characterization of receptors mediating the effects of histamine in many tissues. The many effects of histamine on cardiac physiology have been studied extensively in preparations of

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guinea pig heart (7, 8). Histamine acting at H_1-receptors slows conduction through the atrioventricular node (9) and increases the contractility of left atrial muscle (10, 11). Histamine acting at H_2-receptors increases the rate of contraction of atria (2, 9–13) and increases the contractility of ventricular muscle (9, 11, 13, 14).

There is increasing evidence in support of the hypothesis that the effects of histamine on cardiac contractility are mediated by cAMP (15, 16). Thus, the inotropic effects of histamine on perfused guinea pig heart are potentiated by the phosphodiesterase inhibitor theophylline (17). Histamine and other H_2-receptor agonists increase cAMP levels in heart; this increase in cAMP levels temporally precedes the increase in phosphorylase *a* levels and cardiac contractility that are induced by these agents (14, 17). Furthermore, cAMP derivatives mimic the positive inotropic effects of histamine (and isoproterenol) on cardiac muscle (18).

A histamine-sensitive adenylate cyclase has been demonstrated in cell-free preparations of guinea pig heart (19–23). The effects of various agonists and antagonists (21, 23) on this enzyme indicate that the histamine-binding portion of this adenylate cyclase has pharmacological properties similar to those of an H_2-receptor. These results suggest that the activation of this adenylate cyclase by histamine may be an early step in the sequence of biochemical events through which histamine, acting at H_2-receptors, exerts certain of its physiological effects on cardiac muscle.

A histamine-sensitive adenylate cyclase has also been demonstrated in cell-free preparations of guinea pig brain (24, 25). Quantitative analyses of the effects of H_2-receptor agonists and antagonists on this enzyme suggest that, as in the heart, the histamine-binding portion of this brain adenylate cyclase has pharmacological properties similar to those of the H_2-receptor as defined by physiological experiments on peripheral tissues. However, we have demonstrated (26) that the activation of this brain adenylate cyclase by histamine can also be competitively inhibited by H_1-receptor antagonists and by inhibitors of imidazole-N-methyl transferase (INMT)[1], an enzyme that catalyzes the degradation of histamine. The effects of these compounds on histamine H_2-receptors coupled to adenylate cyclase in peripheral tissues, and on the physiological effects of histamine acting at H_2-receptors in peripheral tissues, have not previously been systematically studied. Consequently, we thought it worthwhile to examine the effects of H_2-receptor antagonists, H_1-receptor antagonists, INMT inhibitors, and various histamine-receptor agonists on the histamine-sensitive adenylate cyclase present in cell-free preparations of guinea pig cardiac ventricular muscle, and to compare the pharmacological properties of this peripheral H_2-receptor with those of the histamine-sensitive adenylate cyclase present in cell-free preparations of guinea pig brain.

MATERIALS

Histamine dihydrochloride, quinacrine dihydrochloride, and EGTA were purchased from Sigma, and 3-isobutyl-1-methylxanthine from Calbiochem. Other compounds used in these experiments were generously provided by pharmaceutical companies: Cimetidine, metiamide, mepyramine maleate, 4-methylhistamine dihydrochloride, 2-methylhistamine dihydrochloride, 2-(2-aminoethyl)pyridine dihydrochloride, and 2-(2-aminoethyl)thiazole dihydrochloride from Smith Kline and French; betazole hydrochloride from Lilly; diphenhydramine hydrochloride and amodiaquine hydrochloride from Parke-Davis; brompheniramine maleate from Robbins; cyclizine hydrochloride, metoprine and etoprine from Burroughs Wellcome; hydroxyzine dihydrochloride from Pfizer; promethazine hydrochloride from Wyeth; clonidine hydrochloride from Boehringer Ingelheim.

METHODS

Adult Hartley guinea pigs (both sexes, weight 150–500 g) were killed by decapitation. The heart was excised, and the ventricles were dissected free from the atria and vasculature. The ventricles were placed

[1] The abbreviations used are: INMT, imidazole-N-methyl transferase; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N′-tetraacetic acid.

in 150 volumes of ice-cold 2 mM Tris maleate (pH 7.8)–2 mM EGTA, and homogenized while on ice with a Polytron, using six 5 sec bursts at 60 sec intervals. This preparation was filtered through two layers of cheesecloth, and was used within 30 min as the enzyme source. In preliminary experiments, the stimulation of adenylate cyclase activity by histamine was the same in this whole homogenate as in a membrane preparation obtained by centrifugation of the whole homogenate at 10,000 × *g* for 15 min; consequently, this centrifugation was omitted in the experiments reported in the present study.

The adenylate cyclase reaction mixture contained the following substances (final concentrations in mM): Tris maleate (pH 7.8), 100; EGTA, 0.6; 3-isobutyl-1-methylxanthine, 1; $MgCl_2$, 2; ATP, 1; GTP, 0.1; plus histamine and test substances as indicated, in a final volume of 500 μl. Following the addition of 50 μl of the homogenate to the reaction mixture minus ATP, each assay tube was vortexed and incubated in an ice-water bath for 10 min. The adenylate cyclase reaction was then initiated by the addition of ATP, carried out for 6 min in a shaking water bath at 30°, and terminated by placing the assay tubes in a hot water bath (93–98°) for 3 min. Fifty microliter aliquots of each sample were assayed in duplicate for cAMP by the method of Brown et al. (27). Protein was determined by the method of Lowry et al. (28). Under these assay conditions, adenylate cyclase activity was linear for at least 15 min and was proportional to added protein up to a final concentration of at least 300 μg/ml.

Inhibition constants (k_i) for compounds that antagonized the stimulation of adenylate cyclase activity by histamine were determined using two independent experimental designs. In one set of experiments, the concentration of antagonist was held constant, and the concentration of histamine was varied. The k_i value was then calculated (29) from the equation:

$$k_i = \frac{I}{\frac{k_a'}{k_a} - 1} \qquad \text{(Eq. 1)}$$

where k_a and k_a' are the concentrations of histamine required to give half-maximal activation of adenylate cyclase in the absence and presence of antagonist, respectively, and I is the concentration of antagonist. In this type of experiment, a parallel displacement of the dose-response curve for histamine by the antagonist is indicative of competitive inhibition at the histamine receptor site.

In another set of experiments, the concentration of histamine (or clonidine) was held constant, and the concentration of antagonist was varied. The k_i value was then calculated (29), assuming competitive inhibition, from the equation:

$$k_i = \frac{IC_{50}}{1 + \frac{S}{k_a}} \qquad \text{(Eq. 2)}$$

where IC_{50} is the concentration of antagonist required to give 50% inhibition of the histamine (or clonidine)-stimulated increase in adenylate cyclase activity, S is the concentration of histamine (or clonidine), and k_a is the concentration of histamine (or clonidine) required for half-maximal activation of adenylate cyclase. The k_a value for histamine used in these calculations was 6 μM.

RESULTS

Effect of histamine and of H_2-receptor antagonists. The effect of various concentrations of histamine on adenylate cyclase activity measured in homogenates of guinea pig cardiac ventricular muscle is shown in Figure 1. In many experiments of this type, the concentration of histamine required for half-maximal activation (k_a) of the enzyme ranged from 5–8 μM; maximal stimulation of adenylate cyclase activity was observed at approximately 100 μM. In various preparations, the maximal activation of cardiac ventricular muscle adenylate cyclase by histamine ranged from 3–6 fold. Figure 1A demonstrates that the H_2-receptor antagonist cimetidine acted as a competitive inhibitor of the activation of adenylate cyclase by histamine. In the presence of 15 μM cimetidine, the concentration of histamine required for half-maximal activation

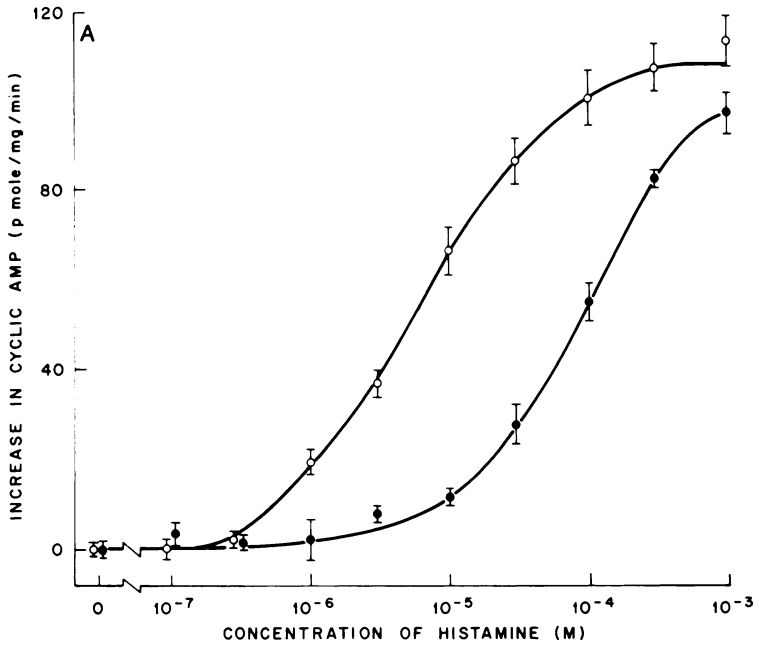


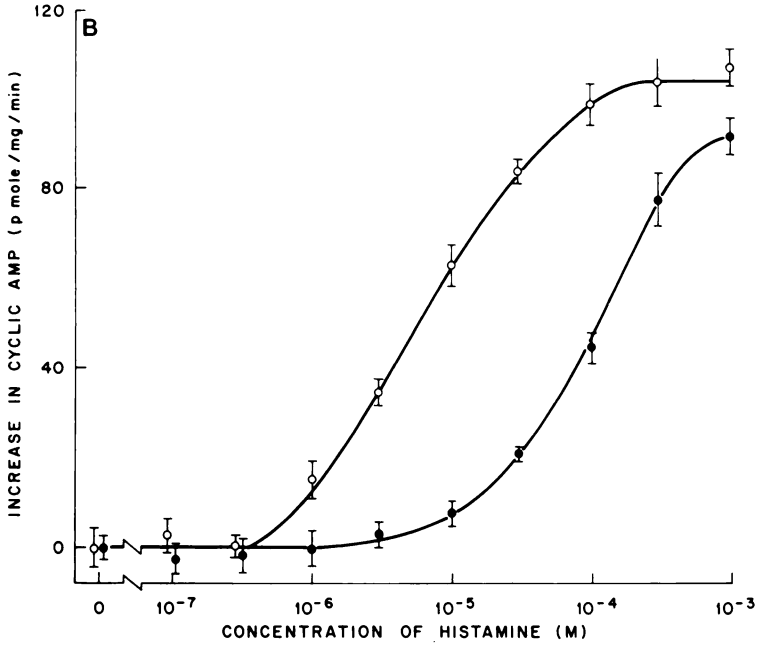
FIG. 1. *Effect of various concentrations of histamine, in the absence* (○) *or presence* (●) *of antagonist, on adenylate cyclase activity in homogenates of guinea pig cardiac ventricular muscle*

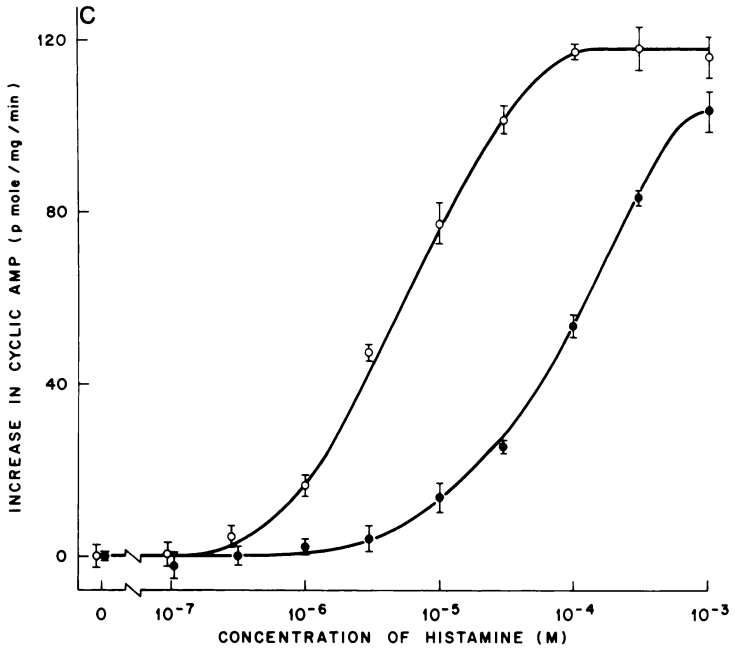
The antagonists and the concentrations used were (1A) cimetidine (15 μM); (1B) promethazine (1.5 μM); and (1C) quinacrine (7.5 μM). In the absence of added histamine or antagonist, adenylate cyclase activity in the experiments shown in Figs. 1A, 1B, and 1C were 33.7 ± 1.2, 27.3 ± 4.3, and 27.0 ± 2.6 pmole cAMP/mg protein/min, respectively. In the absence of added histamine, and in the presence of the given concentration of antagonist, adenylate cyclase activity in the experiments shown in Figs. 1A, 1B, and 1C were 31.3 ± 1.8, 30.5 ± 2.5, and 28.7 ± 1.0 pmole cAMP/mg protein/min, respectively. The increase in cAMP above that observed in the absence of histamine is plotted as a function of the histamine concentration. Values represent the mean ± SEM of six replicate samples in the absence, and three replicate samples in the presence of histamine.

of adenylate cyclase increased from 6 to 100 μM. From these data, the inhibition constant (k_i) of the enzyme for cimetidine was calculated to be 0.96 μM.

The inhibition of histamine-sensitive adenylate cyclase activity by H_2-receptor antagonists was also studied in experiments in which the concentration of antagonist was varied while the concentration of histamine remained constant at 70 μM. Figure 2 illustrates the effects of various concentrations of cimetidine and metiamide on enzyme activity. Low concentrations of each drug blocked the increase in adenylate cyclase activity induced by histamine, while basal enzyme activity was unaffected by the compounds at the concentrations shown. The inhibition constants for the H_2-receptor antagonists cimetidine and metiamide, as determined from experiments of the type shown in Fig. 1A and Fig. 2, are shown in Table 1.

Effect of H_1-receptor antagonists. The effects of six H_1-receptor antagonists on histamine-sensitive adenylate cyclase activity were investigated. As shown in Figure 1B, the phenothiazine H_1-receptor antagonist promethazine acted as a potent competitive inhibitor of the activation of adenylate cyclase by histamine. In the presence of 1.5 μM promethazine, the concentration of histamine required for half-maximal activation of adenylate cyclase increased from 6 to 125 μM. From these data, the inhibition constant of the enzyme for promethazine was calculated to be 0.076 μM. The inhibition of histamine-sensitive adenylate cyclase activity by other H_1-receptor antagonists was studied in further experiments of this type, in which the concentration of





histamine was varied in the absence and presence of a fixed concentration of H_1-receptor antagonist. Every H_1-receptor antagonist studied, including representatives of each of five major chemical classes, was a competitive inhibitor of the activation of adenylate cyclase by histamine. The H_1-receptor antagonists examined included an ethylenediamine (mepyramine), an ethanolamine (diphenhydramine), an alkylamine (brompheniramine), two piperazines (cyclizine, hydroxyzine) and a phenothia-

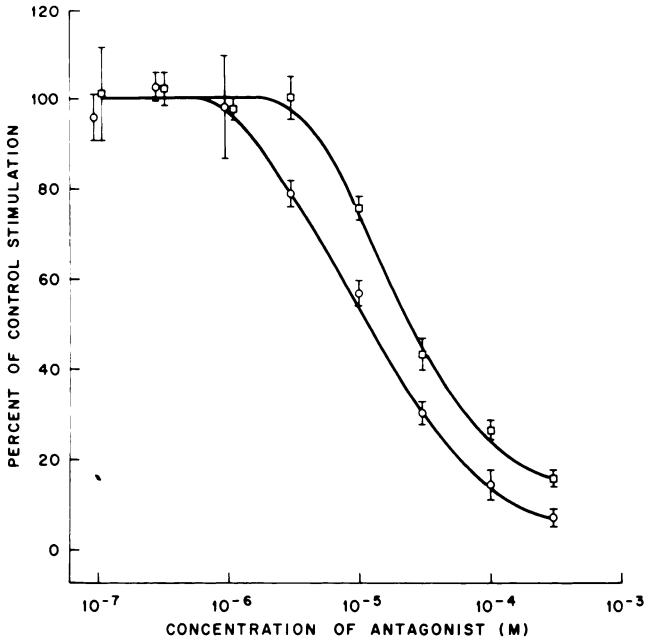


FIG. 2. *Effect of various concentrations of cimetidine* (○) *and metiamide* (□) *on adenylated cyclase activity, measured in the presence of 70 μM histamine, in homogenates of guinea pig cardiac ventricular muscle*

In each experiment, the control stimulation (i.e., the increase in enzyme activity observed in the presence of 70 μM histamine) was defined as 100%, and was based on the measurement of six replicate samples in the absence, and six replicate samples in the presence, of 70 μM histamine. The increase in enzyme activity in the presence of 70 μM histamine, at each concentration of antagonist, is expressed as a percentage of the control stimulation. Each plotted value represents the mean ± SEM of three replicate samples. At the concentrations shown, none of the antagonists appreciably altered adenylate cyclase activity in the absence of added histamine.

zine (promethazine) (Table 1A). Promethazine was considerably more potent as an inhibitor of enzyme activity than any of the other H_1-receptor antagonists tested.

The inhibition of histamine-sensitive adenylate cyclase activity by H_1-receptor antagonists was also studied in experiments in which the concentration of antagonist was varied while the concentration of histamine was held constant at 70 μM (Table 1B). The inhibition constants obtained for each H_1-receptor antagonist, as determined by the two different experimental designs, were in close agreement.

Effect of INMT inhibitors. Several compounds that possess diverse chemical structures and that are known to be inhibitors of INMT (30, 31) were also found to act as competitive inhibitors of histamine-sensitive adenylate cyclase in mammalian brain (26). These compounds also inhibit histamine-sensitive adenylate cyclase activity measured in homogenates of cardiac ventricular muscle. As shown in Figure 1C, the INMT inhibitor quinacrine acted as a competitive inhibitor of the activation of cardiac ventricular muscle adenylate cyclase by histamine. In the presence of 7.5 μM quinacrine, the concentration of histamine required for half-maximal activation of adenylate cyclase increased from 6 to 110 μM. From these data, the inhibition constant (k_i) of the cardiac enzyme for quinacrine was calculated to be 0.43 μM. The inhibition of histamine-sensitive adenylate cyclase activity by other INMT inhibitors was studied in further experiments of this type, in which the concentration of histamine was varied in the absence and presence of a fixed concentration of inhibitor. The INMT inhibitors studied included an acridine derivative (quinacrine), a 4-aminoquinolone (amodiaquine), and two 2,4-diaminopyrimidines (metoprine and etoprine). Each of

TABLE 1

Calculated inhibition constants (k_i) for several different classes of antagonists on histamine-sensitive adenylate cyclase of guinea pig cardiac ventricular muscle

(A) Data are from experiments in which the concentration of histamine was varied, and the concentration of antagonist was held constant. The concentration of antagonist used in each experiment is indicated in parentheses. The inhibition constant (k_i) was calculated according to Equation 1. At the concentrations used in these experiments, the various antagonists had little or no effect on adenylate cyclase activity measured in the absence of added histamine.

Drug	k_a'/k_a	k_i (μM)
H_2-receptor antagonist		
Cimetidine (15 μM)	15.8	0.89
Metiamide (15 μM)	11.4	1.4
H_1-receptor antagonist		
Mepyramine (50 μM)	10.6	5.2
Diphenhydramine (15 μM)	11.4	1.4
Brompheniramine (15 μM)	9.3	1.8
Cyclizine (20 μM)	12.7	1.7
Hydroxyzine (15 μM)	21.7	0.73
Promethazine (1.5 μM)	20.8	0.076
INMT inhibitor		
Quinacrine (7.5 μM)	18.3	0.43
Amodiaquine (100 μM)	13.3	8.1
Metoprine (30 μM)	13.7	2.4
Etoprine (30 μM)	28.7	1.1

(B) Data are from experiments in which the concentration of histamine was held constant at 70 μM, and the concentration of antagonist was varied. The inhibition constant (k_i) was calculated from the observed IC_{50} value according to Equation 2. Basal adenylate cyclase activity was not altered over the range of concentrations of antagonists used to determine the IC_{50} values.

Drug	IC_{50} (μM)	k_i (μM)
H_2-receptor antagonist		
Cimetidine	13	1.0
Metiamide	22	1.7
H_1-receptor antagonist		
Mepyramine	62	4.9
Diphenhydramine	18	1.4
Brompheniramine	20	1.6
Cyclizine	21	1.7
Hydroxyzine	11	0.87
Promethazine	1.0	0.079
INMT inhibitor		
Quinacrine	7.6	0.60
Amodiaquine	110	8.7
Metroprine	38	3.0
Etoprine	21	1.7

these compounds acted as a competitive inhibitor of histamine-sensitive adenylate cyclase activity. Quinacrine was the most potent of these compounds (k_i, 0.43 μM), while the other INMT inhibitors had inhibition constants in the micromolar range (Table 1A).

The inhibition by INMT inhibitors of histamine-sensitive adenylate cyclase activity was also studied in experiments in which the concentration of antagonist was varied while the concentration of histamine was held constant at 70 μM (Table 1B). The inhibition constants for cardiac ventricular muscle adenylate cyclase obtained for each INMT inhibitor, as determined by the two experimental designs, were in close agreement.

Effect of histamine receptor agonists. A number of compounds have recently been developed which act selectively as agonists at H_1- and H_2-receptors in peripheral tissues (2, 3). 4-methylhistamine and betazole are relatively selective H_2-receptor agonists. 2-methylhistamine, 2-(2-aminoethyl)pyridine and 2-(2-aminoethyl)thiazole are relatively selective H_1-receptor agonists, although high concentrations of these compounds will also mimic the physiological effects of histamine acting on peripheral H_2-receptors. The relative potencies of these compounds as agonists on a variety of peripheral H_2-receptor systems have been studied (Table 2). Consequently, it seemed worthwhile to compare the potencies of these compounds as inducers of the physiological responses mediated by peripheral H_2-receptors with their potencies as activators of cardiac ventricular muscle histamine-sensitive adenylate cyclase.

The effects of various concentrations of histamine and of five histamine receptor agonists on adenylate cyclase activity are shown in Figure 3. Histamine itself was the most potent activator of adenylate cyclase. The order of potency among the other agonists was 4-methylhistamine, 2-methylhistamine, 2-(2-aminoethyl)thiazole, betazole, and 2-(2-aminoethyl)pyridine. The relative potencies of these agonists as activators of adenylate cyclase agree well with their relative potencies as inducers of physiological

TABLE 2

Comparison of the relative potencies of histamine receptor agonists on guinea pig cardiac ventricular muscle adenylate cyclase and on physiological responses of several histamine-receptor systems

Histamine receptor agonist	Adenylate cyclase activity		Physiological Response				
			H_2-receptor				H_1-receptor
			Cardiac ventricular contractility	Atrial[f] rate	Uterine relaxation	Gastric acid secretion	Ileum contraction
	k_a (μM)[a]	Ratio[b]	Relative potency				
Histamine	6	(100)	(100)	(100)	(100)	(100)	(100)
4-methylhistamine	28	21.4	25[c]	43	25.3[g]	39[i]	0.2[k]
2-methylhistamine	120	5.0	2[c]	4.4	2.1[g]	2[i]	16.5[k]
Betazole	360	1.7			1[h]	0.5[i], 4.2[j]	0.12[l], 0.06[m]
2-(2-aminoethyl)pyridine	900	0.7	0.9[d]		0.6[h]	0.2[i], 0.7[j]	5.6[l], 3[m]
2-(2-aminoethyl)thiazole	150	4.0				0.3[i]	26[l]
Clonidine	95	6.3	5.9[e]				

[a] Activation constant (k_a) represents the concentration of each agonist causing activation of adenylate cyclase to one-half the maximal level of activity caused by a saturating concentration (300 μM) of histamine.

[b] Ratio = (k_a value for histamine × 100)/(k_a value for histamine receptor agonist).

[c] Relative inotropic effect on isolated perfused guinea pig heart. Reported by Johnson and Mizoguchi (23) based on values estimated from the published graphs of Levi *et al.* (9).

[d] Relative inotropic effect on isolated guinea pig ventricular strips. Reported by Johnson and Mizoguchi (23) based on values estimated from the published graphs of Perez de Gracia and De Mello (36).

[e] Relative inotropic effect on isolated perfused guinea pig heart. Reported by Csongrady and Kobinger (32).

[f] Relative chronotropic effect on isolated guinea pig atria. Reported by Black *et al.* (2).

[g] Relative potency for inhibition of electrically-induced contraction of isolated rat uterus. Reported by Black *et al.* (2).

[h] Relative potency for inhibition of electrically-induced contraction of isolated rat uterus. Reported by Ash and Schild (1).

[i] Relative potency for stimulation of gastric acid secretion in the anesthetized rat. Reported by Durant *et al.* (3).

[j] Relative potency for stimulation of gastric acid secretion in the anesthetized rat. Reported by Ash and Schild (1).

[k] Relative potency for stimulation of contraction of guinea pig ileum. Reported by Black *et al.* (2).

[l] Relative potency for stimulation of contraction of guinea pig ileum. Reported by Durant *et al.* (3).

[m] Relative potency for stimulation of contraction of guinea pig ileum. Reported by Ash and Schild (1).

responses mediated by H_2-receptors (Table 2).

We attempted to determine whether each of these agonists was exerting its stimulatory effects on adenylate cyclase activity by acting on the same receptor as histamine. If this were the case, and if one assumes that histamine itself causes maximal activation of the H_2-receptor, then one would expect (a) that the increase in adenylate cyclase activity produced by a saturating concentration of each agonist would not exceed that produced by a saturating concentration of histamine, and (b) that the increase in adenylate cyclase activity produced by a high concentration of each agonist plus a saturating concentration of histamine would not exceed that produced by a saturating concentration of histamine alone. The effects of high concentrations of various agonists on adenylate cyclase activity, in the absence and presence of a saturating concentration of histamine (300 μM), are shown in Table 3. In no case did the maximal stimulation of adenylate cyclase activity by the histamine receptor agonist exceed the maximal stimulation of enzyme activity by histamine. The stimulation of adenylate cyclase activity produced by 3 mM 2-(2-aminoethyl)pyridine and by 3 mM betazole was less than that produced by a saturating concentration of

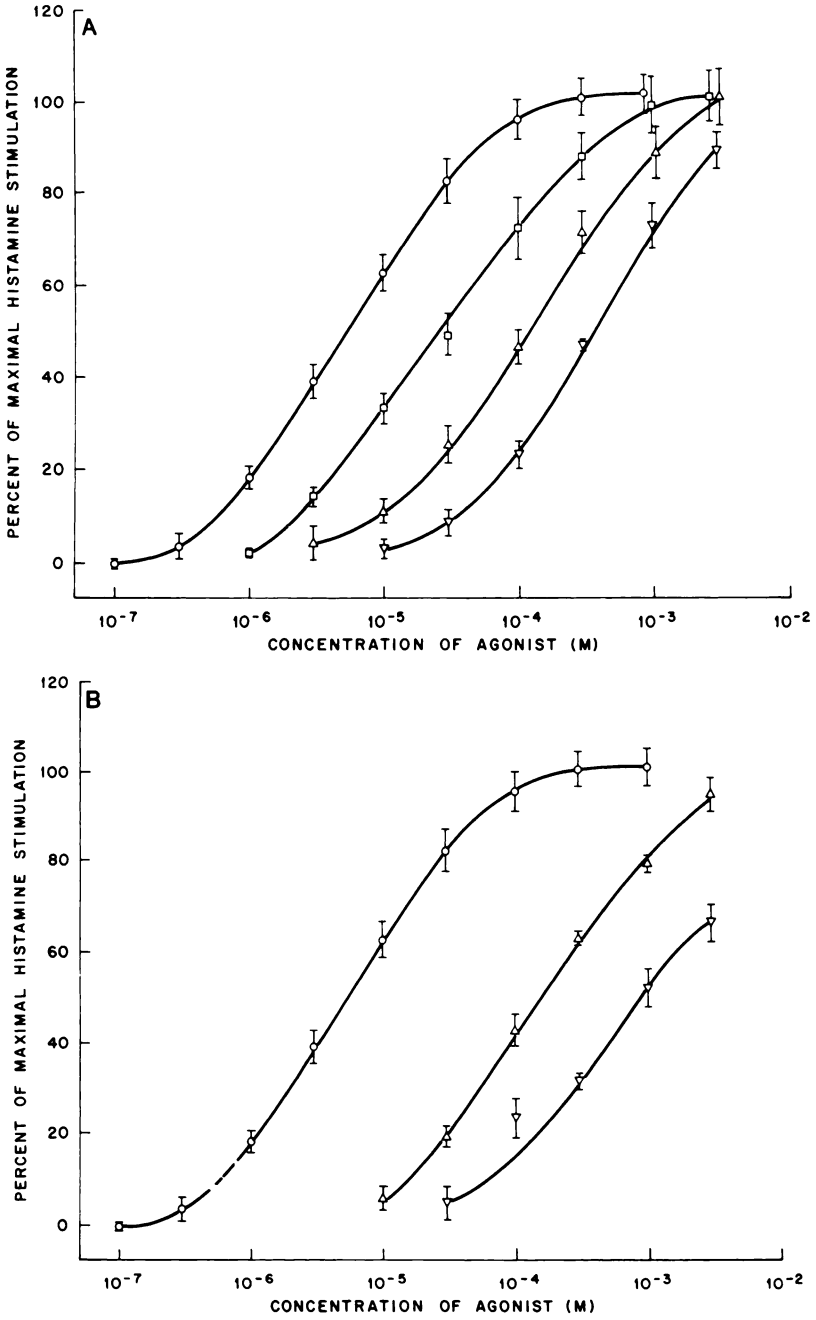


FIG. 3. *Effect of various concentrations of histamine and of histamine receptor agonists on adenylate cyclase activity in homogenates of guinea pig cardiac ventricular muscle*

The increase in adenylate cyclase activity at a saturating concentration of histamine (300 μM) is defined as 100%, and is based on the measurement of six replicate samples in the absence, and of six replicate samples in the presence, of 300 μM histamine. For each given concentration of histamine or histamine receptor agonist, the increase in enzyme activity is expressed as a percentage of the increase in adenylate cyclase activity due to 300 μM histamine. Each plotted value represents the mean ± SEM of three replicate samples. (3A) histamine (○), 4-methylhistamine (□), 2-methylhistamine (△), betazole (▽); (3B) histamine (○), 2-(2-aminoethyl)-thiazole (△), 2-(2-aminoethyl)pyridine (▽).

TABLE 3

Effect of histamine receptor agonists on adenylate cyclase activity in homogenates of guinea pig cardiac ventricular muscle

Values in the absence of any test substance represent the mean ± SEM for nine replicate samples in the absence, and nine replicate samples in the presence, of 300 μM histamine. Values in the presence of test substance (concentrations indicated in parentheses) represent the mean ± SEM for three replicate samples.

Test substance	Adenylate cyclase activity	
	− Histamine	+ Histamine (300 μM)
	(pmole cAMP/mg protein/min)	
None	51.4 ± 1.4	179.3 ± 2.3[a]
4-methylhistamine (300 μM)	176.8 ± 4.8	187.0 ± 5.0
2-methylhistamine (1 mM)	174.6 ± 2.3	185.9 ± 8.8
2-(2-aminoethyl)pyridine (3 mM)	140.9 ± 1.7[c]	189.1 ± 9.0[b]
2-(2-aminoethyl)thiazole (3 mM)	180.8 ± 5.2	184.1 ± 4.6
Betazole (3 mM)	154.0 ± 4.6[c]	184.1 ± 16.5

[a] Activity in the presence of histamine was significantly higher ($p < 0.005$) than that in the absence of histamine.

[b] Activity in the presence of histamine and test substance was significantly higher ($p < 0.025$) than that in the presence of test substance alone.

[c] Activity was significantly different ($p < 0.005$) than that in the presence of histamine and absence of test substance.

histamine; however, as is evident from Figure 3, this concentration of each of these agonists was not quite sufficient to saturate the histamine receptor coupled to adenylate cyclase. High concentrations of the five agonists tested, in the presence of a saturating concentration of histamine, did not produce greater stimulation of adenylate cyclase activity than did a saturating concentration of histamine alone. These results suggest that these agonists and histamine may cause their stimulatory effects on adenylate cyclase activity by acting on the same receptor.

Effect of clonidine. The centrally acting antihypertensive drug clonidine has been reported to increase cardiac contractility (32, 33), phosphorylase *a* (33), and cAMP levels (33) in guinea pig heart. These effects of clonidine are blocked by the H_2-receptor antagonist burimamide. Clonidine has also been reported to increase cAMP levels by activation of an H_2-receptor in guinea pig stomach (34) and brain (35). We investigated the effect of clonidine on adenylate cyclase activity measured in homogenates of guinea pig cardiac ventricular muscle. As shown in Figure 4, low concentrations of clonidine stimulated enzyme activity. The concentration of clonidine required for stimulation of adenylate cyclase activity to one-half the extent produced by saturating concentrations of histamine was 95 μM. However, higher concentrations of clonidine did not stimulate adenylate cyclase activity to the same extent as did saturating concentrations of histamine. Thus, the increase in enzyme activity produced by a maximally effective concentration of clonidine (1 mM) was only 78% of that produced by 300 μM histamine.

To determine whether clonidine was exerting its stimulatory effects on adenylate cyclase activity by acting on the same receptor as histamine, we compared the stimulation of enzyme activity produced by 1 mM clonidine plus 300 μM histamine with that produced by 300 μM histamine alone. In each of three experiments, the activation of adenylate cyclase by the combination of clonidine plus histamine was only slightly (about 10–15%) greater than that produced by histamine alone. The effects of the H_2-receptor antagonist cimetidine on the stimulation of adenylate cyclase activity by clonidine was also examined. As shown in Figure 5, high concentrations of cimetidine completely blocked the activation of adenylate cyclase produced by clonidine. The concentration of cimetidine required to give half-maximal inhibition of the stimulation of enzyme activity produced by 1 mM clonidine was 12 μM. From this IC_{50} value, and assuming that 95 μM represents the k_a value of the enzyme for clonidine, the inhibition constant for cimetidine inhibition of the stimulation of adenylate cyclase activity produced by clonidine was calculated to be 1.04 μM. This value is in good agreement with the inhibition constant for cimetidine of 0.89 μM obtained using histamine as the

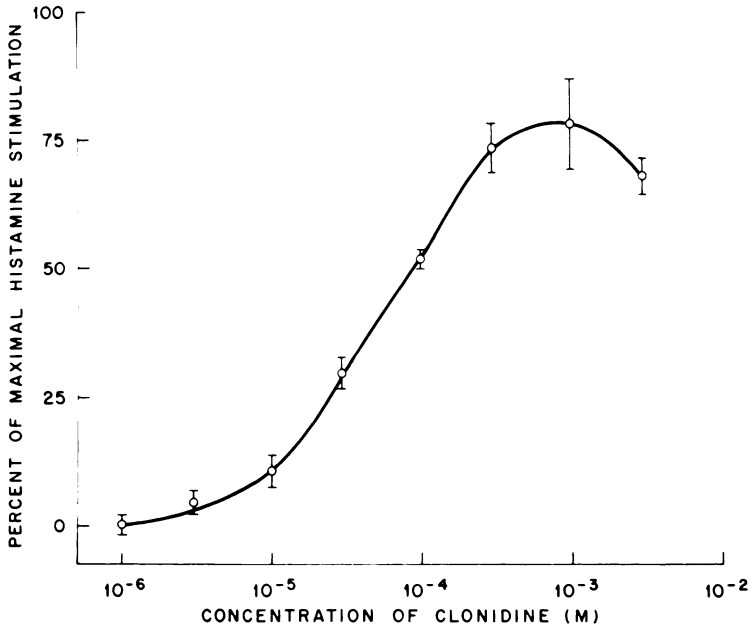


FIG. 4. *Effect of various concentrations of clonidine on adenylate cyclase activity in homogenates of guinea pig cardiac ventricular muscle*

For each concentration of clonidine, the increase in enzyme activity is expressed as a percentage of the increase in adenylate cyclase activity caused by a saturating concentration (300 μM) of histamine. The maximal stimulation of adenylate cyclase activity by histamine (4.3-fold), defined as 100%, was determined by measurement of six replicate samples in the absence, and of six replicate samples in the presence of 300 μM histamine. Each plotted value represents the mean ± SEM of three replicate samples.

activator of adenylate cyclase. In other experiments, cimetidine was shown to inhibit the activation of adenylate cyclase by clonidine in a competitive manner. These results suggest that clonidine produces most of its stimulatory effect on adenylate cyclase activity by interacting with the same receptor as does histamine.

DISCUSSION

The results of previous studies (21, 23) and of the present study support the conclusion that the histamine-binding portion of the histamine-sensitive adenylate cyclase present in homogenates of cardiac ventricular muscle possesses pharmacological properties similar to those of the H_2-receptor as defined by physiological experiments on peripheral tissues. Thus, the reported inhibition constants (5, 6) for the H_2-receptor antagonists metiamide and cimetidine are 0.92 μM and 0.79 μM, respectively, for inhibition of the chronotropic effects of histamine on guinea pig atria, and are 0.75 μM and 0.81 μM, respectively, for inhibition of the relaxant effects of histamine on the electrically-induced contractions of rat uterus. These inhibition constants, determined in physiological experiments on H_2-receptor systems, agree well with the inhibition constants for metiamide and cimetidine of 1.4 μM and 0.89 μM, respectively, as inhibitors of the stimulation by histamine of cardiac ventricular muscle adenylate cyclase. Similarly, as shown in Table 2, the relative potencies of a variety of compounds as agonists of physiological responses mediated by H_2-receptors (but not H_1-receptors) agree very well with their relative potencies as activators of adenylate cyclase in homogenates of cardiac ventricular muscle. (One apparent exception is 2-(2-aminoethyl)thiazole, which appears to be more potent relative to histamine in stimulating cardiac ventricular muscle adenylate cyclase than in stimulating gastric acid secretion. However, the agonist effects of this compound on other H_2-receptor systems

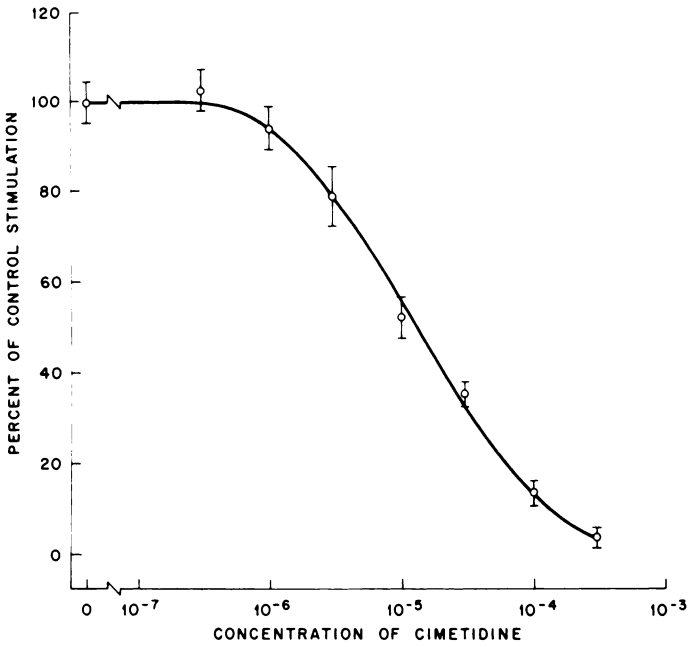


FIG. 5. *Effect of various concentrations of cimetidine on adenylate cyclase activity measured in the presence of 1 mM clonidine, in homogenates of guinea pig cardiac ventricular muscle*

The increase in enzyme activity in the presence of 1 mM clonidine, at each concentration of cimetidine, is expressed as a percentage of the control stimulation (i.e., the stimulation due to 1 mM clonidine in the absence of cimetidine). Values represent the mean ± SEM of six replicate samples in the absence, and three replicate samples in the presence of cimetidine.

have not been reported.) Thus, the pharmacological properties of the histamine-sensitive adenylate cyclase present in homogenates of guinea pig cardiac ventricular muscle resemble those of an H_2-receptor.

There is conflicting experimental evidence concerning the efficacy of blockade by H_1-receptor antagonists of the physiological effects of histamine mediated by H_2-receptors in the heart. Trendelenburg (37) observed that high concentrations of the H_1-receptor antagonist mepyramine antagonized the stimulation by histamine of the rate of contraction of guinea pig atria. The inhibition constant for this effect of mepyramine was found to be 5 μM, which is in excellent agreement with the inhibition constant for mepyramine of 5.2 μM found in the present study of cardiac ventricular muscle adenylate cyclase. These values are ten thousand times higher than the inhibition constant of 0.5 nM found (38) for inhibition of a response to histamine mediated by H_1-receptors (contraction of guinea pig ileum). Similarly, it has been found that high concentrations of the H_1-receptor antagonist diphenhydramine blocked the stimulation by histamine of the rate of contraction of guinea pig atria (39), and that high concentrations of the H_1-receptor antagonist tripelennamine blocked the effects of histamine on cardiac contractility (20). However, other investigators (7, 40) have failed to observe effects of H_1-receptor antagonists on the stimulation by histamine of cardiac rate or contractility. It has also been found that low concentrations of the phenothiazine H_1-receptor antagonist promethazine blocked the increase in cardiac rate (40, 41), contractility (11, 40, 41) and cAMP levels (41) induced by histamine. However, the nature of this inhibition by promethazine was found to be irreversible (40) or noncompetitive (11, 41). Other studies have failed to demonstrate an effect of promethazine on the responses due to histamine of cardiac atrial rate (9, 13) or ventricular contractility (13). The reason for

these conflicting results concerning the effects of H_1-receptor antagonists on the physiological effects of histamine mediated by H_2-receptors in the heart is unclear, but may be due, in part, to differences in the concentrations of histamine and of the H_1-receptor antagonists used in the various physiological experiments. Moreover, the H_1-receptor antagonists also possess local anesthetic properties at high concentrations (42); this action greatly complicates the task of interpreting experiments with these compounds involving physiological responses (e.g., changes in atrial rate and ventricular contractility) that are dependent on alterations of membrane permeability to specific ions.

The reported effects of H_1-receptor antagonists on histamine-sensitive adenylate cyclase in cardiac muscle are also conflicting. It has been reported that the activation of cardiac adenylate cyclase by histamine can be blocked by high concentrations of diphenhydramine (19) and mepyramine (22). McNeill and Muschek (20) demonstrated inhibition of cardiac histamine-sensitive adenylate cyclase activity by high concentrations of tripelennamine and diphenhydramine; however these authors claimed that the nature of the inhibition of the enzyme by diphenhydramine was noncompetitive. Recently, Johnson and Mizoguchi (23) found that the activation of cardiac ventricular muscle adenylate cyclase by histamine was inhibited by high concentrations of tripelennamine in a competitive manner. In agreement with these authors, we have found that representatives of five major chemical classes of H_1-receptor antagonists acted as competitive inhibitors of histamine-sensitive adenylate cyclase activity measured in homogenates of guinea pig cardiac ventricle. Many of the H_1-receptor antagonists tested had inhibition constants for the enzyme in the same range as the H_2-receptor antagonists metiamide and cimetidine. In addition, we have found that the H_1-receptor antagonist promethazine was more than 10 times as potent as the H_2-receptor antagonist cimetidine as an inhibitor of histamine-sensitive adenylate cyclase activity in guinea pig cardiac ventricular muscle. Similar cross-reactivity of H_1-receptor antagonists on H_2-receptors coupled to adenylate cyclase has been observed in studies of the histamine-sensitive adenylate cyclases in gastric mucosa (43, 44) and brain (26).

It should be emphasized that the H_1-receptor antagonists are much less potent as inhibitors of H_2-receptors coupled to adenylate cyclase than as inhibitors of the physiological effects of histamine mediated by H_1-receptors. For example, the inhibition constants for mepyramine, diphenhydramine, and promethazine are 0.44 nM, 7.2 nM, and 1.2 nM, respectively, for inhibition of the histamine-induced contraction of guinea pig ileum (4), while the inhibition constants for these compounds on histamine-sensitive adenylate cyclase in homogenates of guinea pig cardiac ventricular muscle are 5.2 μM, 1.4 μM, and .076 μM, respectively. Thus, mepyramine, diphenhydramine, and promethazine are approximately 12,000, 200, and 60 times more potent, respectively, as inhibitors of peripheral H_1-receptors than as inhibitors of cardiac ventricular muscle histamine-sensitive adenylate cyclase. These results clearly indicate that the pharmacological properties of the histamine-binding portion of this adenylate cyclase present in cardiac ventricular muscle are not those of an H_1-receptor.

The results of the present study indicate that a group of INMT inhibitors, which were found to inhibit histamine-sensitive adenylate cyclase activity in homogenates of brain (26), also inhibit histamine-sensitive adenylate cyclase activity in homogenates of cardiac ventricular muscle. Since these compounds block H_2-receptors as well as INMT, they could be expected to have dual actions on any physiological event mediated by histamine acting on an H_2-receptor: the event might be potentiated by the inhibition of histamine catabolism due to blockade of INMT, but the event might be blocked due to the H_2-receptor antagonist properties of these compounds. In fact, it has been shown that the INMT inhibitor amodiaquine potentiates the stimulation of gastric acid secretion produced by histamine (45). Thus, in this system, the properties of amodiaquine as an INMT inhibitor

appear to predominate over its properties as an H_2-receptor antagonist in determining the net physiological effect of this compound on an H_2-receptor mediated event.

It is of interest that the H_2-receptor antagonists, H_1-receptor antagonists and INMT inhibitors share a common site of action, both as inhibitors of cardiac muscle histamine-sensitive adenylate cyclase and as inhibitors of the histamine catabolic enzyme INMT (30, 31, 46, 47). These observations suggest that the histamine binding portion of the histamine-sensitive adenylate cyclase and the active site of INMT possess certain features in common, enabling each to be inhibited by the same set of compounds.

In the previous (26) and present studies of the histamine-sensitive adenylate cyclases present in homogenates of guinea pig brain and heart, the enzyme activities derived from these two tissues were measured under identical assay conditions of buffer, pH, metal chelators, cofactors, and substrates. Consequently, one would expect that if the histamine-sensitive adenylate cyclases derived from guinea pig brain and heart were identical, the inhibition constant

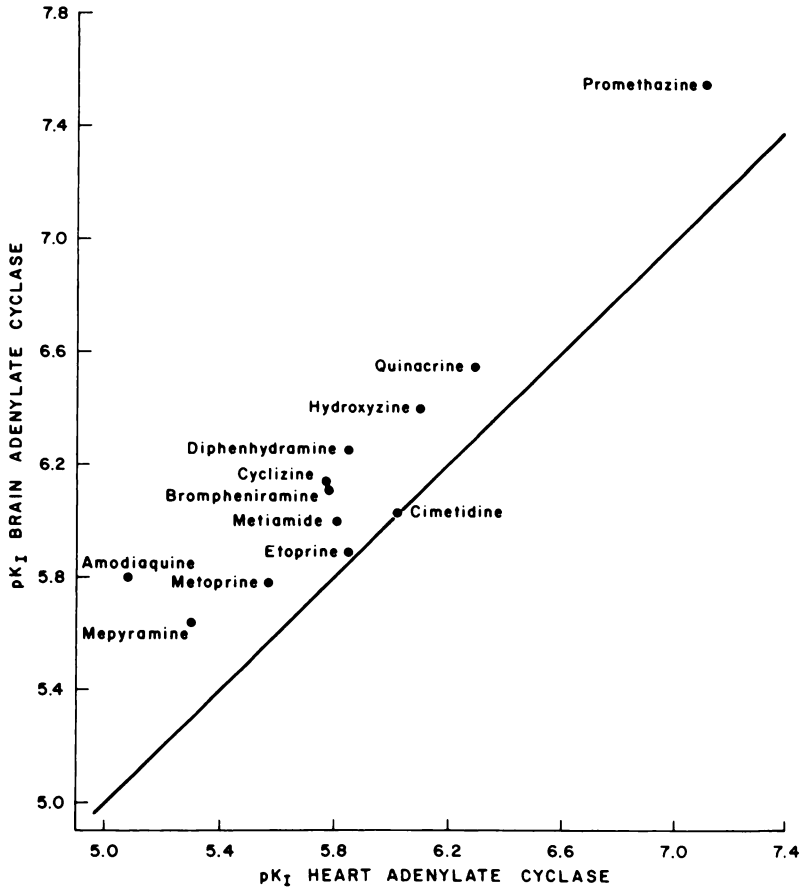


FIG. 6. *A comparison of the inhibition constants of 12 compounds on histamine-sensitive adenylate cyclase derived from guinea pig heart and brain*

The abscissa of each point represents the negative logarithm of the inhibition constant (pK_i) of the indicated compound on the enzyme derived from guinea pig cardiac ventricular muscle, and the ordinate represents the pK_i of that compound on the enzyme derived from the dorsal hippocampus from guinea pig brain. If the antagonist-binding portions of the adenylate cyclases derived from the two sources were identical, any receptor antagonist would, in theory, have identical inhibition constants for the enzyme from the two sources, and any such pair of inhibition constants would fall along the line drawn.

obtained for each antagonist would be independent of the enzyme source. In certain cases, this was not observed. In Figure 6, the inhibition constants for 12 competitive inhibitors of histamine-sensitive adenylate cyclase activity were plotted; for each point, the abscissa represents the pk_i for the enzyme derived from guinea pig cardiac ventricular muscle, and the ordinate represents the pk_i for the enzyme derived from the dorsal hippocampus of guinea pig brain. The inhibition constants for certain compounds (cimetidine and etoprine) were identical for the enzymes derived from the two sources. However, certain other compounds (e.g., mepyramine, diphenhydramine, promethazine) were more than twice as potent as inhibitors of the brain enzyme than of the heart enzyme, and the INMT inhibitor amodiaquine was more than five times as potent as an inhibitor of the brain enzyme than of the heart enzyme. None of the 12 compounds was more potent as an inhibitor of the enzyme derived from heart than of the enzyme derived from brain. These observed differences were highly reproducible in repeated experiments. The reasons for these apparent differences in the pharmacological properties of the two enzymes remain uncertain. It is possible that the protein moieties of the two histamine receptors coupled to adenylate cyclase are identical, but that, in the presence of the different phospholipid environments in membranes from brain and heart, the resulting pharmacological properties of the histamine-receptor-adenylate cyclase complex are different. Alternatively, it is possible that there exist two distinct isozymes for the H_2-receptor, one present in brain and one present in heart. These receptor isozymes, each functionally coupled to an adenylate cyclase, might possess somewhat different pharmacological properties. A search for more selective antagonists of the histamine-sensitive adenylate cyclases, as well as the development of methods to isolate and purify H_2-receptors, may help to elucidate the apparent pharmacological heterogeneity of H_2-receptors in different tissues.

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