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Pharmacological Characterization of Histamine Receptors Mediating the Stimulation of Cyclic AMP Accumulation in Slices from Guinea-Pig Hippocampus

J. M. PALACIOS, M. GARBARG, G. BARBIN, AND J. C. SCHWARTZ

Unité 109 de Neurobiologie, Centre Paul Broca de l'I. N. S. E. R. M., 2ter rue d'Alesia, 75014 Paris, France

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SUMMARY

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A histamine-sensitive adenylate cyclase presenting characteristics very similar to typical H_2-receptors has recently been demonstrated in cell-free brain preparations, whereas the stimulation of cyclic AMP accumulation elicited by histamine in cerebral slices has been postulated to involve both H_1- and H_2-receptors. We have reinvestigated the pharmacological properties of histamine receptors mediating the stimulation of cyclic AMP in guinea-pig hippocampal slices by determining the relative potencies of several agonists and the apparent affinities of antagonists. Metiamide, a H_2-blocker, antagonizes in a competitive manner the stimulation elicited by histamine (or 2-thiazolylethylamine, a predominantly H_1-agonist) with a pA_2 value of 6, corresponding to an interaction with a single population of typical H_2-receptors. The relative potencies of two H_2-agonists (dimaprit and 4-methylhistamine) are consistent with this view. However other data suggest the involvement of an heterogeneous population of histamine receptors. First, the relative potencies of two H_1-agonists (2-thiazolylethylamine and 2-methylhistamine) in hippocampal slices are intermediate between corresponding values on homogeneous populations of H_1- and H_2-receptors, respectively. Furthermore in the presence of moderate concentrations (5 nM to 0.1 μM) of mepyramine, an H_1-antagonist, the response to histamine is modified in a complex fashion: the responses to histamine in low concentrations remain unaltered whereas those to the amine in concentrations above 10 μM are competitively antagonized. Analysis of these data by Schild plot or by a computer program reveals two components in the response to histamine: a first one (insensitive to mepyramine) with an EC_{50} of 6μM and a second one with an EC_{50} of 14 μM for the amine (antagonized by mepyramine with a pA_2 of 8.2). The participation of each component to the maximal response is approximately 50%. In addition, a series of H_1-antagonists inhibit the response to 100 μM histamine with apparent K_B 5 toward the second component which appear to be in the same range as those toward typical H_1-receptors. Furthermore the supramaximal response to dimaprit, a pure H_2-agonist, is progressively elevated in the presence of 2-thiazolylethylamine, a predominantly H_1-agonist, in increasing concentrations. In these conditions the relative potency of 2-thiazolylethylamine becomes comparable with the value for typical H_1-receptors. This additive response is blocked by 0.1 μM mepyramine while the response to dimaprit alone is not affected. It is concluded that the response to histamine in brain slices involves the stimulation of both typical H_2-receptors and receptors closely similar to (or identical with) H_1-receptors, which appear to be activated in a sequential manner.

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INTRODUCTION

Ten years ago Kakiuchi and Rall (1) showed that histamine was one of the most powerful agents in stimulating cyclic AMP accumulation in brain slices. In 1966, Ash and Schild (2) provided some pharmacological evidence that the actions of histamine on various biological systems from peripheral tissues were mediated by at least two distinct classes of receptors. The work of Black *et al.* (3) supported this evidence by clearly establishing the existence of H_1- and H_2-receptors and providing specific agents to characterize them.

Since that time much work has been devoted to the pharmacological characterization of histamine receptors mediating the stimulation of cyclic AMP formation in brain tissues (for reviews, see references 4–6) but a rather confusing picture has emerged.

Recently two groups of investigators have independently demonstrated the existence of a histamine-sensitive adenylate cyclase in cell-free preparations from guinea-pig brain and have shown that the receptor involved had characteristics very similar to typical H_2-receptors (7, 8). For instance, H_2-antagonists competitively block histamine stimulation with pA_2 values almost identical to those found on typical H_2-systems from peripheral organs, and H_2-agonists stimulate the cyclase with relative potencies close to those exhibited on these systems (8). In addition the actions of H_1-agonists or antagonists occurred only at high concentrations, i.e., their apparent affinities were consistent with an interaction with a single population of H_2-receptors.

However, when the same pharmacological tools are used to characterize the action of histamine on cyclic AMP accumulation in brain slices a different, less clear picture emerges.

In guinea-pig hippocampal slices Chasin *et al.* (9) observed that H_1-antagonists partially blocked the response to histamine. Baudry *et al.* (10) showed that either a H_1-antagonist, mepyramine, or a H_2-antagonist, metiamide, inhibited the histamine response by approximately 50%. The response was completely antagonized only in the presence of the two agents; the ratio of the IC_{50} of the two antagonists was close to that reported for peripheral systems containing pure populations of H_1- or H_2-receptors. In addition, 4-methylhistamine, a relatively specific H_2-agonist (3), elicited a response that was inhibited by metiamide but not by mepyramine. The conclusion was that both H_1- and H_2-receptors mediate the histamine response on slices from guinea-pig cortex. Rogers *et al.* (11) reached the same conclusion by employing a series of antagonists of H_1- and H_2-receptors and suggested that H_1 receptors mediate about 20% of the response to histamine in slices from guinea-pig cortex but around 50% in hippocampal slices. Based on the ability of both H_1- and H_2-agonists, 2-thiazolylethylamine and 4-methylhistamine, to stimulate cyclic AMP accumulation in slices from guinea-pig hippocampus and cortex, Dismukes *et al.* (12) also inferred that two classes of receptors were present; the fact that metiamide antagonized the action of the H_1-agonist was explained by the low affinity of the latter as well as by the relative nonspecificity of the antagonist. In contrast with the results obtained with slices from guinea-pig brain, Nahorski *et al.* (13) suggested that the response on slices from chick brain was mediated exclusively by H_2-receptors: both 10 μM metiamide and 100 μM mepyramine had antagonist properties, but only the action of metiamide could be overcome by raising the histamine concentration.

However, none of the above studies with brain slices determined the dissociation constants of the histamine antagonists (or the relative potencies of agonists) precisely nor did they compare them with corresponding values on typical H_1- and H_2-receptors. Hence it can be concluded that, in contrast with cell-free preparations in which a single population of H_2-receptors has been clearly identified, the identity of receptors mediating the effects of histamine on cyclic AMP accumulation in slices is still to be accurately characterized. We have attempted this characterization on guinea-pig hippocampal slices, in view of the high responsiveness of this preparation to histamine.

MATERIALS AND METHODS

Male Hartley guinea-pigs (300 g) were used in all experiments. The animals were killed by decapitation and hippocampus quickly dissected on ice. Slices (250 μm thick) from at least four animals, were prepared employing a McIlwain tissue slicer. The pooled slices were incubated 30 min at 37° in Krebs-Ringer bicarbonate medium (50 ml per g of tissue) under a constant stream of O_2:CO_2 (95:5) in a Dubnoff metabolic shaker. At the end of this preincubation, slices were washed twice with fresh medium and 200 μl aliquots of the suspension distributed in incubation tubes. The tubes were gassed with O_2:CO_2 and incubated again for 10 min at 37°. The different agents were added at the beginning of the incubation. Since cyclic AMP accumulation elicited by histamine reaches a plateau at 10 min and remains constant at least up to 20 min, an incubation time of 15 min was employed at 37° and under agitation, as described previously (10). Incubations were terminated by sonication of the slices and heating of the homogenates at 95° for 8 min. Cyclic AMP concentration was determined by the protein binding assay method of Brown *et al.* (14). Protein concentration was measured in each incubation tube by the Folin procedure (15). Results are presented as pmol cyclic AMP per mg protein formed during the incubation time, or as percentages of the maximal stimulation elicited by agonists.

Concentration-response curves were fitted by hand or by employing the computer method of Parker and Waud (16) or an iterative computing method developed in our laboratory.[1] This method allows the determination of the 6 parameters of the following equation according to the least squares:

$$y = \frac{V_1 \cdot x^{n_1}}{EC_{50_1}{}^{n_1} \cdot x^{n_1}} + \frac{V_2 \cdot x^{n_2}}{EC_{50_2}{}^{n_2} \cdot x^{n_2}}$$

The equation describes biphasic curves, i.e., curves that are the sum of two components. In this equation, y represents the percentage of the maximal response for the agonist concentration x; V_1 and V_2 are the maximal participation to the total response corresponding to each component; EC_{50_1} and EC_{50_2} are the agonist concentrations inducing half-maximal response in each component; n_1 and n_2 are the Hill coefficients of each component.

[^{3}H]cyclic AMP (8-[^{3}H]adenosine 3′,5′ cyclic phosphate, 30 Ci/mmol) was obtained from the Radiochemical Centre (Amersham, U.K.). Dimaprit, 4-methylhistamine, 2-methylhistamine, 2-thiazolylethylamine and metiamide were generously provided by Dr. M. E. Parsons (The Research Institute, Smith, Kline and French Laboratories, U.K.). The H_1-antagonists, mepyramine, triprolidine, cyclizine, promethazine, D-brompheniramine, and diphenhydramine, were generously provided by the Burroughs-Wellcome and Specia companies.

RESULTS

Studies with agonists. The effects of 4-methylhistamine and dimaprit (S-[3-(N,N-dimethylamino) propyl]isothiourea), two H_2-agonists (3, 17), and of 2-thiazolylethylamine and 2-methylhistamine, two H_1-agonists (3, 18), on cyclic AMP accumulation in slices from guinea-pig hippocampus were compared with those of histamine. Typical concentration-response curves for these agonists are presented in Fig. 1. The data are expressed as percentages of maximal stimulation elicited by each compound in order to define the EC_{50} value and to establish the equipotent molar ratio for the determination of its "relative potency," (RP) i.e.,

$$RP = \frac{EC_{50}\ \text{histamine}}{EC_{50}\ \text{agonist}} \times 100$$

The results of these calculations are shown in Table 1 and are compared with corresponding data from the literature for histamine-stimulated adenylate cyclase in hippocampal homogenates (8) and well-characterized H_1- and H_2-receptors in peripheral systems: contraction in the isolated guinea-pig ileum (H_1) and gastric secretion in the rat (H_2)(3, 17–19).

In addition, clear differences in the intrinsic activities (20) of these agents are found as shown by the values of the maxi-

[1] Malfroy *et al.*, manuscript in preparation.

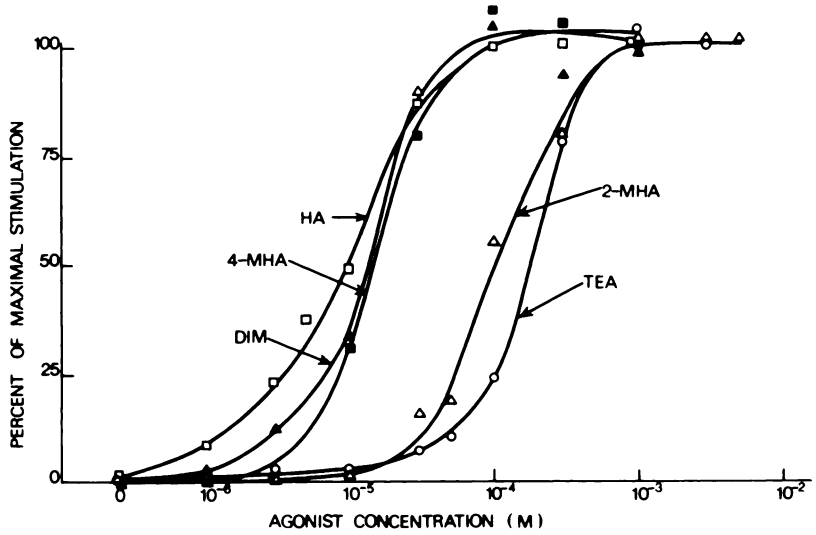

FIG. 1. *Concentration-response curves of the cyclic AMP accumulation induced by histamine, H_1- and H_2-histamine receptor agonists in slices from guinea-pig hippocampus*

Slices were incubated for 15 min. Histamine (HA) (□); dimaprit (DIM) (▲); 4-methylhistamine (4-MHA) (■); 2-methylhistamine (2-MHA) (△); 2-thiazolylethylamine (TEA) (○) were added at the beginning of the incubation. Each point represents the mean data from 6 incubations from two separate experiments; variation was less than 10% of mean values. Results are expressed as percent of maximal stimulation elicited by each agent. The maximal values (in pmoles of cyclic AMP per mg protein) are 93 ± 6 for histamine; 18 ± 1 for dimaprit; 22 ± 2 for 4-methylhistamine; 32 ± 1 for 2-methylhistamine; and 66 ± 4 for 2-thiazolylethylamine. The mean basal level was 5.4 ± 0.3.

TABLE 1

Comparison of the "relative potencies" of histamine agonists on the accumulation of cyclic AMP in hippocampal slices and on other systems

Relative potencies were calculated according to the equation

$$R.P. = (EC_{50} \text{ histamine}/EC_{50} \text{ agonist}) \times 100$$

The EC_{50} values for agonists were calculated by log-probit analysis of the concentration-response curve of each compound on hippocampal slices.

Agonist	Cyclic AMP accumulation in hippocampal slices	Adenylate cyclase in hippocampal homogenates	Gastric secretion in rat (H_2)	Ileum contraction in guinea-pig (H_1)
Histamine	100	100[a]	100[b]	100[b]
Dimaprit	67	219[a]	19.5[c]	0.0001[c]
4-Methylhistamine	67	58[a]	39[b]	0.3[b]
2-Methylhistamine	12	12[a]	2[b]	16.5[b]
2-Thiazolylethylamine	7	—	0.3[d]	26[d]

[a] Green *et al.* (8).
[b] Johnson & Mizoguchi (19).
[c] Parsons *et al.* (17).
[d] Durant *et al.* (18).

mal stimulation reported in the legend of Fig. 1.

Studies with antagonists. The cyclic AMP accumulation induced by histamine was evaluated in the presence of increasing concentrations of the H_2-antagonist metiamide (21). The rightward shift of the concentration-response curves without modification of the maximal responses (Fig. 2) indicates that metiamide competitively inhibits histamine-activated cyclic AMP accumulation. Furthermore, when metiamide inhibition is analyzed by means of Schild plot (22) (inset of Fig. 2), the slope

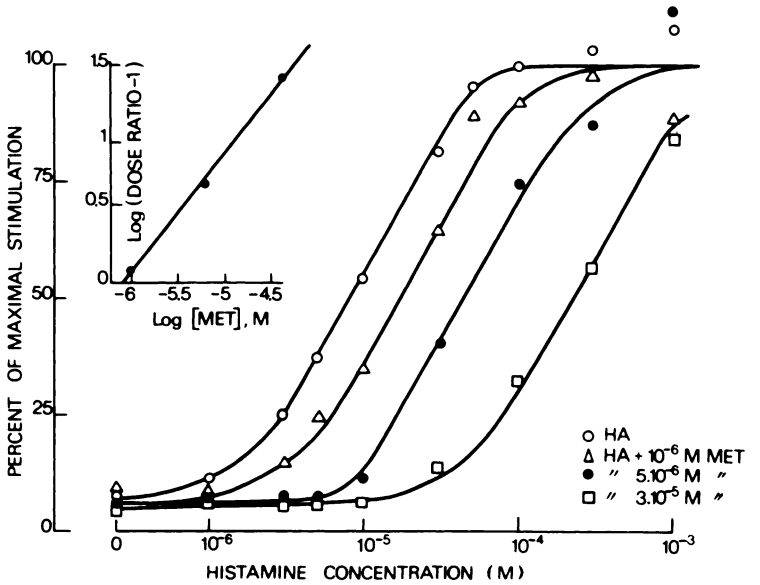

FIG. 2. *Inhibition by metiamide of the histamine-induced accumulation of cyclic AMP in slices from guinea-pig hippocampus*

Slices were incubated in the presence of various concentrations of histamine (HA) added at the beginning of the incubation alone (○) or together with 10^{-6} M (△), 5×10^{-6} M (●) or 3×10^{-5} M (□) metiamide (MET). Points represent the means of data from 6 incubations from two separate experiments. Data varied less than 10% of the means. The results are expressed as percentage of the maximal response to histamine. Basal level of cyclic AMP was not significantly modified by the antagonist at any of the studied concentrations. The inset represents the Schild plot of the same data. Dose ratios were estimated graphically from the parallel displacement of straight portions of the concentration-response curves. The pA_2 value determined by linear regression was 6.04.

of the line is not different from unity, indicating simple competitive antagonism. The pA_2 value, i.e., the negative logarithm of the molar concentration of the antagonist that reduces the effect of a given agonist concentration to that of half this concentration (22) was calculated and a value of 6.04 obtained. Metiamide also inhibited competitively the cyclic AMP accumulation elicited by 2-thiazolylethylamine (Fig. 3). When the data were analyzed by Schild plot, the pA_2 value for metiamide was in the same range as that found for this compound against histamine.

Mepyramine is an H_1-antagonist with a high affinity (apparent dissociation constant [K_B]:0.1 nM) for typical H_1-receptors (22). We have studied the effects of this compound, in concentrations ranging from 50 pM to 1 μM, on the histamine-induced accumulation of cyclic AMP (Fig. 4). Mepyramine was ineffective at 50 pM and 0.5 nM. At concentrations ranging between 5 nM and 0.1 μM, a complex action was observed: mepyramine did not affect cyclic AMP accumulation induced by low concentrations of histamine (up to 5 μM) but inhibited the effects of higher concentrations (10 μM to 1 mM) of the agonist in a competitive manner. In the presence of 1 μM mepyramine, the effects of low concentrations of histamine were also unchanged but the effects of histamine in higher concentrations (up to 1 mM) were inhibited in a insurmountable fashion. The Schild plot for mepyramine inhibition (inset of Fig. 4), established from the parallel displacement of the upper part of the curves, yielded a line with a slope equal to unity and an intercept at the abscissa axis at a value (pA_2) of 8.2.

In view of their complexity, the concentration-response curves to histamine in the presence of mepyramine were also analyzed by a computed iterative curve-fitting method developed (see MATERIALS AND METHODS), after that of Parker and Waud (16). The data fit a bimodal curve, indicating the presence of two components in the

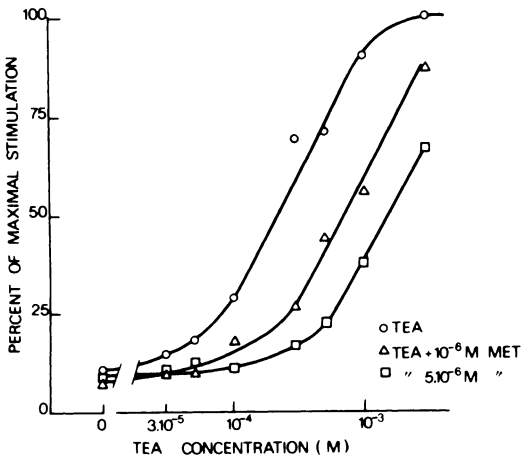

FIG. 3. *Inhibition by metiamide of the 2-thiazolylethylamine induced accumulation of cyclic AMP in slices from guinea-pig hippocampus*

Slices were incubated in the presence of 2-thiazolylethylamine (TEA) alone (○) or in combination with 10^{-6} M (△) or 5×10^{-6} M (□) metiamide (MET). Points represent the means of data from 3 separate incubations that varied less than 8%. Results are expressed as percentage of the maximal response elicited by 2-thiazolylethylamine. Basal level of cyclic AMP was not significantly modified by the antagonist at the concentrations employed.

histamine-induced accumulation of cyclic AMP. The EC_{50} of the first segment (6 μM) did not vary in the presence of mepyramine, while that of the second component (14 μM) increased with the antagonist concentration (Table 2). Both components seem to follow Michaelis-Menten kinetics as the Hill coefficients (data not shown) were not significantly different from unity. The relative contribution of each component of the response to the maximal response remained constant in the presence of mepyramine, and was approximately 50% for each. The K_B value of mepyramine for the competitively antagonized component is 3.5 nM, corresponding to a pA_2 of 8.4 ($pA_2 = -\log K_B$), a value in close agreement with that derived from Schild plot analysis (Fig. 4).

The specificity of the action of mepyramine in moderate concentrations, on only one type of receptor was confirmed by using the highly selective H_2-agonist, dimaprit. Complete concentration-response curves for dimaprit in the presence of concentrations of mepyramine ranging from 50 nM to 0.5 μM (concentrations that produced inhibition of the histamine response) were constructed (Table 3). No inhibition of the dimaprit-induced cyclic AMP accumulation was found at any antagonist concentration.

As another criterion for the involvement of H_1-receptors, the potency of a series of H_1-blockers ("classical" antihistamines) was investigated. When slices were incubated in the presence of 0.1 mM histamine and increasing concentrations of these compounds, a progressive inhibition of the response to histamine was observed (Fig. 5). For some compounds, i.e., mepyramine, triprolidine, and promethazine, a clear plateau was observed, corresponding approximately to a 50% inhibition. Assuming that this plateau corresponded to the total inhibition of the second component of the response to histamine, the concentration required for half-maximal inhibition of this component, i.e., 25% inhibition of the maximal response to histamine, was determined for each compound.

Based on these IC_{50} values, the apparent K_B of the compounds for the second component were calculated, assuming a competitive inhibition. These apparent K_B values agree reasonably well with those reported for the same compounds on the guinea-pig ileum (23, 24), a typical H_1-system (Table 4).

Effects of the combination of dimaprit and 2-thiazolylethylamine on cyclic AMP accumulation. If both H_1- and H_2-receptors are present in hippocampal slices, an additive response must be expected when H_1- and H_2-agonists are added simultaneously.

To test this hypothesis, hippocampal slices were incubated in the presence of 2-thiazolylethylamine, either alone or in combination with a supramaximal concentration of dimaprit (Fig. 6). In these conditions a supraadditive response was observed for concentrations between 10 and 100 μM. However the maximal response to the H_1-agonist was not significantly altered in the presence of 100 μM dimaprit. Hence, in the presence of dimaprit, the EC_{50} of 2-thiazolyl-ethylamine is shifted from 0.2 mM to 60

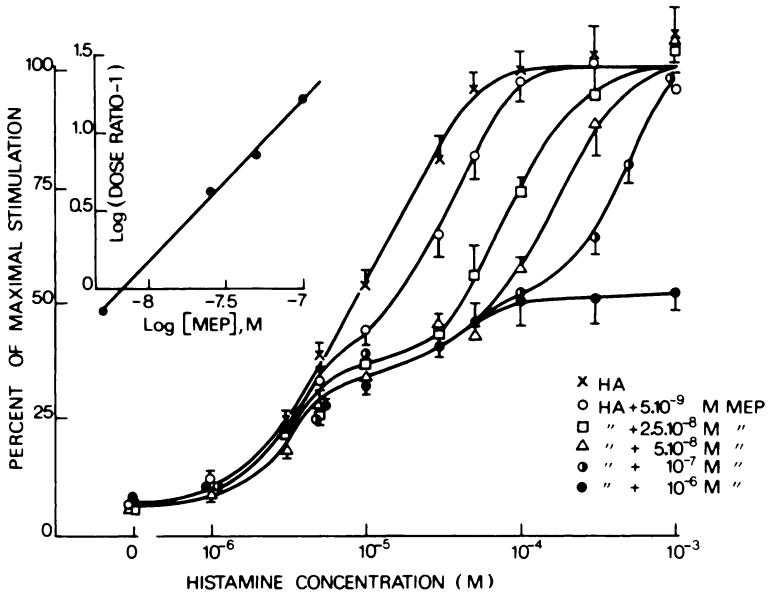

FIG. 4. *Inhibition by mepyramine of the histamine-induced accumulation of cyclic AMP in slices from guinea-pig hippocampus*

Histamine (HA) was added at the beginning of the incubation alone (×) or together with 5×10^{-9} M (○); 2.5×10^{-8} M (□); 5×10^{-8} M (△); 10^{-7} M (◑); and 10^{-6} M (●) mepyramine (MEP). Basal levels of cyclic AMP were not significantly altered by mepyramine at any concentration. Each point represents the mean ± S.E.M. of results from 6–12 incubations performed in separate experiments. Results are expressed as percentage of the maximal stimulation elicited by histamine. In the inset the Schild plot of these data is represented. Dose ratios were obtained graphically from the parallel displacement of upper parts of the curves (corresponding to 60–80% of the maximal stimulation).

μM. When the relative potency to histamine of 2-thiazolylethylamine in the presence of dimaprit was calculated, a value of 23% was obtained, which is close to that found on a peripheral homogeneous H_1-receptor system (see Table 1). Moreover, while the response to dimaprit alone was not affected by 0.1 μM mepyramine (Table 3), the effect of 2-thiazolylethylamine was inhibited by the H_1-blocker.

DISCUSSION

Ideally, the "taxonomy of receptors," (25) mediating a given biological response is based on findings from studies with the use of specific agonists and antagonists recognizing selectively a class of receptors, but this condition is rarely met. In the case of the two classes of histamine receptors, the pharmacological tools presently available consist of a highly specific agonist of the H_2-receptors, dimaprit (17), and various agonists and antagonists of H_1- and H_2-receptors with lower specificity but presenting nevertheless a reasonably large difference in affinity for the two classes (25). Therefore a conclusive identification of histamine receptors mediating the stimulation of cyclic AMP formation in brain tissues requires the establishment of the apparent dissociation constants (K_B) of antagonists as well as, to a certain extent, the relative potency of agonists (8).

In a cell-free preparation, i.e., the homogenate from guinea-pig hippocampus, it is clear that, based on these criteria, the stimulation of adenylate cyclase induced by histamine (7, 8) is mediated by an homogeneous population of receptors representing the same characteristics as typical H_2-histamine receptors (3). Several of our findings could be interpreted as evidence that it is also the case for the stimulation of cyclic AMP accumulation in the slice preparation.

For instance, dimaprit stimulates cyclic AMP accumulation in slices in the same

range of concentration as does histamine: its potency relative to histamine is 67%, a value close to those found on homogeneous populations of H_2-receptors (Table 1). In contrast, its relative potency on a typical H_1-system is 0.0001% (17). The same conclusion can be made as regard the action of another H_2-agonist, 4-methylhistamine, although its specificity is inferior to that of dimaprit (Table 1).

Furthermore, the effects of metiamide, a specific H_2-antagonist, give additional support to the concept that the response to histamine in slices is mediated by a homogeneous population of typical H_2-receptors. It produces a parallel displacement in the concentration-response curves to histamine and its effects are surmountable by increasing the agonist concentration, indicating a competitive inhibition. The Schild plot analysis of metiamide effects gives a straight line with a slope equal to unity, confirming this type of inhibition. A value of 6 is obtained for the pA_2, in close agreement with the values for metiamide in uterus and atrium (21), two well-characterized H_2-receptor systems. The inhibition by metiamide of the response to 2-thiazolylethylamine (Fig. 3) could be interpreted in the same way. Hence all the above data suggest the exclusive involvement of typical H_2-receptors in the histamine response in hippocampal slices, as is the case for those linked to an adenylate cyclase in cell-free hippocampal preparations.

However other data are not in agreement with this conclusion and suggest that H_1-receptors may also be involved. Thus, a first indication is given when two agonists with a relatively specific action on H_1-receptors are used. Both 2-thiazolylethylamine and 2-methylhistamine elicit a response in slices, with a relative potency to histamine intermediate between the corresponding values reported for these compounds acting on homogeneous populations of H_1- (see ref. 3) and H_2- (see ref. 18) receptors. Our data are in relatively good agreement with those of Dismukes *et al.* (12) and Nahorski *et al.* (26), although complete concentration-response curves were not constructed in all cases. Even more interesting were the results obtained by

TABLE 2

Parameters of concentration-response curves to histamine in the presence of mepyramine

The data from Fig. 3, analyzed by a computed iterative curve-fitting method (see MATERIAL AND METHODS), were best fit to a two-component curve, the parameters of which are reported. The values varied less than 8.6%. If competitive inhibition of mepyramine for the second component is assumed, the apparent K_B was 3.5 nM.

Mepyramine concentration	First component		Second component	
	Participation to maximal response	EC_{50}	Participation to maximal response	EC_{50}
M	%	μM	%	μM
0	56	6.8	55	14
5×10^{-9}	55	6.2	52	33
2.5×10^{-8}	50	6.2	57	110
5×10^{-8}	50	6.2	62	210
3×10^{-7}	49	3.8	54	407

TABLE 3

Effect of mepyramine on dimaprit stimulated accumulation of cyclic AMP in hippocampal slices

Concentration-response curves of the cyclic AMP accumulation induced by dimaprit in slices from guinea-pig hippocampus were constructed in the absence or in the presence of several concentrations of mepyramine in two different experiments. Mepyramine did not affect the basal level of cyclic AMP. EC_{50} and maximal stimulation were determined by analyzing the data, by the method of Parker and Waud (16).

Response to dimaprit	Mepyramine concentration					
	Experiment 1			Experiment 2		
	0	5×10^{-8} M	10^{-7} M	0	2.5×10^{-7} M	5×10^{-7} M
EC_{50} ($\times 10^{-5}$ M)	0.7 ± 0.3	0.6 ± 0.4	2.6 ± 1	1.6 ± 1.2	2.7 ± 2.3	2.7 ± 1.8
Maximal stimulation (pmol cyclic AMP per mg of protein)	12 ± 1	12.2	14 ± 1	20 ± 3	22 ± 4	24 ± 4

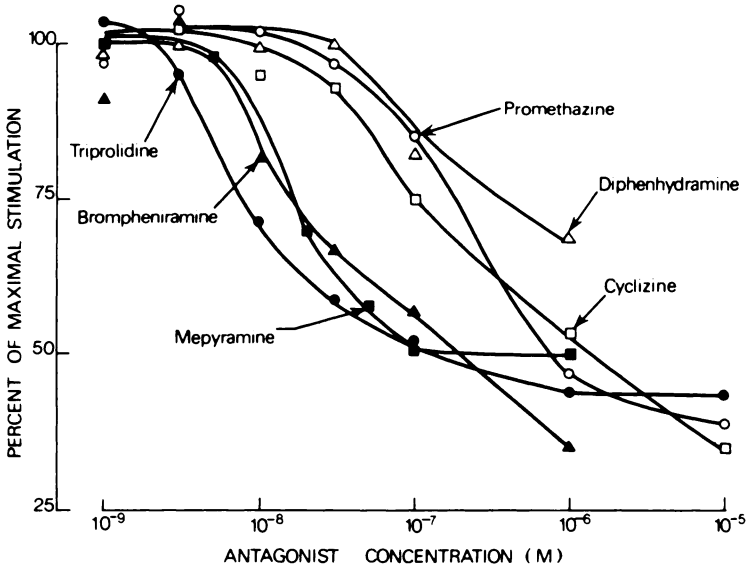

FIG. 5. *Effect of various H_1-antagonists on the histamine-induced accumulation of cyclic AMP in slices from guinea-pig hippocampus*

Slices were incubated in the presence of 10^{-4} M histamine in the presence of increasing concentrations of triprolidine (●) brompheniramine (▲) mepyramine (■) promethazine (○) diphenhydramine (△) and cyclizine (□). Points are the means of data from 3-6 separate incubations. The stimulation elicited by 0.1 mM histamine in the absence of antagonist is defined as maximal stimulation and results for each antagonist are expressed as percentages of this value.

TABLE 4

Comparison of K_B values of several H_1-antagonists on the histamine-induced accumulation of cyclic AMP in hippocampal slices and on a typical H_1-system

Antagonist	K_B[a] (hippocampal slices)	K_B[b] (guinea-pig ileum)
	M	*M*
Triprolidine	10^{-9}	1.2×10^{-10}
Mepyramine	2×10^{-9}	5×10^{-10}
Brompheniramine	2×10^{-9}	1.1×10^{-9}[c]
Cyclizine	1.2×10^{-8}	1.3×10^{-8}
Promethazine	2.5×10^{-8}	1.2×10^{-9}
Diphenhydramine	5×10^{-8}	7.2×10^{-9}

[a] K_B values were calculated using the equation $K_B = IC_{50}/(1 + S/K_A)$. IC_{50} refers to the antagonist concentration that produces a 25% inhibition of the maximal stimulation elicited by 10^{-4} M histamine, i.e., agonist concentration producing 50% inhibition of the second component of histamine concentration-response curves. These values were obtained from data plotted in Fig. 5. S is the concentration of histamine (10^{-4} M) and K_A is the histamine concentration that gives half-maximal stimulation of the second component, i.e., 75% of the maximal response to histamine.

[b] Data collected by Triggle and Triggle (23).

[c] Calculated from Roth (24).

constructing concentration-response curves for histamine in the presence of specific H_1-antagonist, mepyramine (Fig. 4). The complexity of the interaction of this agent was indicated by the fact that the responses to concentrations of histamine below 5 μM were not significantly affected, while the responses to higher concentrations were clearly inhibited, suggesting two distinct components in the response to histamine. The first component is represented by the response to low histamine concentrations and characterized by its insensitivity to mepyramine; in contrast, the second component is characterized by the antagonism exerted by mepyramine, which produces a parallel shift of the upper part of the concentration-response curves, without change in the maximal response. The competitive nature of the inhibitory effect of mepyramine was confirmed by analyzing the data with the Schild plot: a straight line with a slope equal to unity was obtained giving a pA_2 value of 8.2. This value is far from 5.3, the pA_2 for mepyramine acting either on an homogeneous population of typical H_2-re-

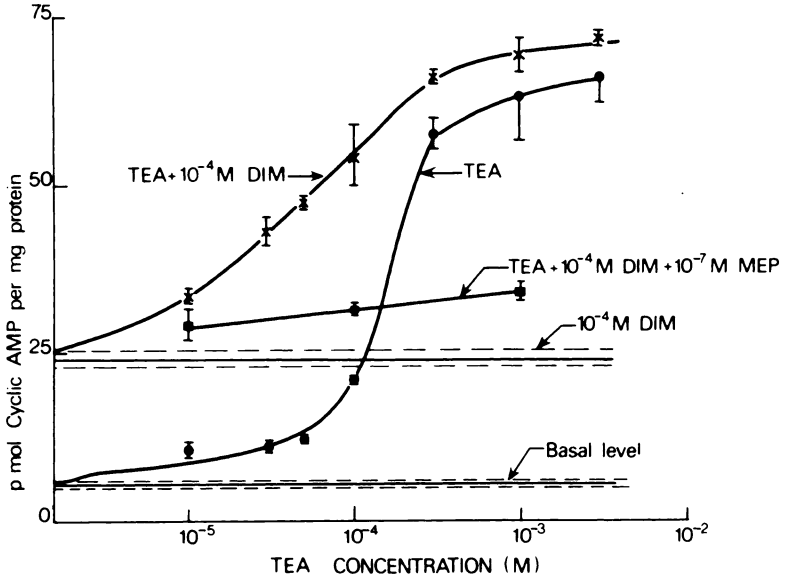

FIG. 6. *Cyclic AMP accumulation in slices from guinea-pig hippocampus induced by 2-thiazolylethylamine in the presence of dimaprit*

2-Thiazolylethylamine (TEA) in varying concentrations was added either alone (●) or together with 10^{-4} M dimaprit (×) (DIM). The effects of 10^{-7} M mepyramine (MEP) on the stimulation elicited by the combination of TEA and DIM was also investigated (■); mepyramine added 10 min before the two agonists. Basal level of cyclic AMP and stimulation elicited by 10^{-4} M DIM are represented by horizontal lines. Each point represents the mean ± S.E.M. of data from 3 separate incubations.

ceptors (27) or on the adenylate cyclase from guinea-pig hippocampus (8), i.e., there is a 1,000 fold-difference in apparent affinity, rendering an inhibition of the H_2-receptor-mediated response in slices unlikely.

The bimodal nature of the response to histamine, as revealed by mepyramine, is confirmed when the data are analyzed by an iterative computing method[1] derived from that of Parker and Waud (16). This mathematical treatment shows that the data are best fitted to a two-component curve with an approximately 50% participation of each to the maximal response and EC_{50} of 6 μM and 14 μM, respectively. The small difference between these EC_{50}s explains the failure to detect the two components in the concentration-response curve to histamine in the absence of mepyramine. In addition, this mathematical treatment confirms the competitive nature of mepyramine inhibition (progressive increase in EC_{50} without change in the participation to maximal response, see Table 2) as well as the apparent affinity of mepyramine for the second component determined by Schild plot.

That mepyramine actions are not mediated by blockade of H_2-receptors is also substantiated by the absence of significant change in either the EC_{50} or the maximal response to dimaprit (Table 3) at antagonist concentrations up to 0.5 μM that already inhibit the histamine response (Fig. 4). A nonspecific action of mepyramine, related either to H_2-receptor blockade or to its local anesthetic properties (28), could occur at higher concentrations of this agent, as suggested by Green *et al.* (8) and Nahorski *et al.* (26) and possibly demonstrated by the apparently insurmountable inhibition at 1 μM (Fig. 4).

Moreover the stimulation of cyclic AMP accumulation elicited by 2-thiazolylethylamine in the presence of a supramaximal concentration of dimaprit also reveals the second component, as well as its sensitivity to mepyramine (Fig. 6).

Is this second component of histamine action mediated by typical H_1-receptors? If

this is the case, the apparent affinity of H_1-antagonists in hippocampal slices should be comparable to that found in well-defined H_1-systems. In the case of mepyramine, the pA_2 obtained from the Schild plot (Fig. 4) is 8.2, a value corresponding to a dissociation constant of 6.3 nM; a similar K_B value for the second component is determined by the computed iterative curve-fitting method (Table 2) or by considering the inhibition produced by the antagonist in increasing concentrations (Fig. 5, Table 4). On many peripheral systems containing an homogeneous population of typical H_1-receptors, the pA_2 for mepyramine is between 9.1 and 9.4 (22), while on typical H_2-receptors its pA_2 is 5.1–5.3 (8, 27). Thus it is clear that the apparent affinity of mepyramine for the second component of histamine action is much higher than that of the drug on typical H_2-receptors while it is close to that found in typical H_1-receptors. The same conclusion holds for the apparent K_B of a series of H_1-receptor antagonists, which are in a rather good agreement with those reported in the guinea-pig ileum (Table 4). Taken together, these results indicate that the second component of histamine action is probably mediated by typical H_1-receptors. The slight (4–5 fold) difference in the dissociation constants of some H_1-antagonists, in hippocampal slices as compared to ileum, might be due to the fact that our calculations depend on the approximate estimation of the relative sizes of the two components of histamine response. However it cannot be excluded that H_1-receptors in brain present some slight differences with the corresponding ones in peripheral tissues.

As both H_1- and H_2-receptors appear to be involved in the stimulation of cyclic AMP accumulation elicited by histamine in hippocampal slices, one could ask whether the responses mediated by each class of receptors are independent or are linked in some way. The observations that metiamide, a specific H_2-antagonist, completely inhibits the response to histamine with a pA_2 corresponding to a homogeneous population of H_2-receptors, while mepyramine blocks only a fraction of this response, suggests a sequential mechanism in the actions of the agonist: it appears that the response mediated by H_1-receptors depends upon H_2-receptor stimulation.

This would account for the monophasic response to histamine in the presence of metiamide, contrasted with the biphasic one in the presence of mepyramine. This would also explain the action of H_1-agonists: since both 2-thiazolylethylamine and 2-methylhistamine possess significant H_2-agonist activity, the stimulation they elicit is probably mediated by activation of H_2-receptors followed by that of H_1-receptors. Thus, their relative potencies are intermediate between the corresponding values on homogeneous populations of H_1- and H_2-receptors (Table 1). This interpretation is strengthened by the observation that the relative potency of 2-thiazolylethylamine becomes almost identical to its relative potency on typical H_1-receptors when H_2-receptors are stimulated by a supramaximal concentration of dimaprit, a pure H_2-agonist. It would also account for the complete inhibition by metiamide of the effect of the H_1-agonists (Fig. 3), also reported by others (12, 26).

The necessity of a preliminary stimulation of H_2-receptors to observe the H_1-receptor-mediated effect also explains why responses to H_1-agonists can be found only in slices from brain regions in which an H_2-mediated response is observed (29). Furthermore the relative importance of the responses mediated by H_1- and H_2-receptors, respectively, appears to be the same in the various areas of the guinea-pig brain in which histamine stimulates cyclic AMP accumulation: in all cases 50 nM mepyramine inhibits by approximately 50% the response to 0.1 mM histamine.[2]

The involvement of H_1-receptors in the stimulation of cyclic AMP accumulation in slices is evident from our studies, but the exact mechanism of their action is not entirely clear at the present time. While a typical H_2-receptor appears to be directly coupled to an adenylate cyclase both in brain (7, 8) and in peripheral tissues (19, 30, 31), such evidence is lacking for H_1-receptors. Therefore one could speculate that the

[2] Manuscript in preparation.

H_1-receptor mediated effect is an indirect one resulting from i) an increased efficiency of the coupling between the H_2-receptor and the catalytic unit, ii) a decreased breakdown of cyclic AMP synthesized in the slices via H_2-receptor stimulation, iii) the release of a messenger producing one of the above effects, a likely candidate for this role being adenosine (12). It could also be hypothesized that histamine receptors in brain all belong to the H_2-class but that, upon stimulation, a fraction of the receptor molecules undergoes a conformational change leading to the appearance of characteristics of H_1-receptors. A temperature-dependent interconversion of H_1- and H_2-receptors has already been suggested (32). Whatever the exact mechanism involved, our observations suggest that H_1-receptors are involved in the action of histamine on target-cells in brain and that the well-known sedative actions (28) of most classical antihistamines, i.e., H_1-antagonists, could be due to an inhibition of histaminergic transmission.

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