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Characterization of histamine receptors mediating the stimulation of cyclic AMP accumulation in rabbit cerebral cortical slices

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1 The characteristics of histamine-stimulated adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation in slices of rabbit cerebral cortex have been investigated.

2 The selective H_2-receptor antagonists, cimetidine, tiotidine, metiamide and ranitidine appeared to antagonize the stimulation of cyclic AMP accumulation elicited by histamine in a competitive manner consistent with an interaction with histamine H_2-receptors.

3 The H_1-receptor antagonist mepyramine (0.8 μM) produced only a weak inhibition of the response to histamine. The inhibition appeared to be non-competitive producing a decrease in the maximal response with little effect on the EC_{50} value.

4 The specific H_2-receptor agonist, impromidine, produced a maximum response of only 31 ± 2% of that obtained with histamine. Studies with histamine and impromidine in combination indicated that impromidine was not acting as a partial agonist. 2-Thiazolylethylamine, a selective H_1-agonist, produced only a weak response ($EC_{50} \sim$ 1 mM) yielding a relative potency with respect to histamine (= 100) of 2.5.

5 In the presence of a supramaximal concentration of impromidine, histamine and 2-thiazolylethylamine further elevated the response to impromidine. In these conditions the relative potency of 2-thiazolylethylamine was increased to 59 (histamine = 100), a value which was comparable with that reported for H_1-receptor-mediated contractions of guinea-pig ileum.

6 The H_1-receptor antagonists mepyramine, promethazine, triprolidine and chlorpheniramine competitively antagonized the potentiation of impromidine-stimulated cyclic AMP accumulation elicited by histamine and 2-thiazolylethylamine in rabbit cerebral cortex without affecting the response to impromidine alone. (+)-Chlorpheniramine was some 150 fold more potent than the (−)-isomer in this respect.

7 Histamine and adenosine in combination had a much greater than additive effect on the accumulation of cyclic AMP in rabbit cerebral cortical slices. The potentiation of the adenosine response could be partially but not completely antagonized by either cimetidine or mepyramine.

8 In the presence of H_2-receptor blockade with 0.02 mM tiotidine, histamine elicited a significant potentiation (EC_{50} 44 μM) of the response to adenosine. This response was antagonized competitively by mepyramine yielding a K_B value of 0.05 μM similar to that obtained from inhibition of the potentiation of impromidine-stimulated accumulation of cyclic AMP (0.02 μM).

9 These results suggest that there are two components in the response to histamine in rabbit cerebral cortical slices. The first component appears to be mediated by histamine H_2-receptors while the second, mepyramine-sensitive, component has some of the characteristics of an H_1-receptor mediated response and requires prior stimulation of adenosine- or H_2-receptors to produce its effect.

Introduction

Histamine is one of the most powerful agents in stimulating adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation in the mammalian central nervous system (Daly, 1977). In cell-free preparations from guinea-pig cerebral cortex and hippocampus, several workers have demonstrated the presence of a

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histamine-sensitive adenylate cyclase (Hegstrand *et al.*, 1976; Green *et al.*, 1977; Green, 1983) which appears to be linked exclusively to histamine H_2-receptors. In contrast, in slices of guinea-pig cerebral cortex and hippocampus, the effect of histamine on cyclic AMP accumulation appears also to be mediated by histamine H_1-receptors. The H_1-actions, in contrast with the direct H_2-action, appear to be indirect and to require prior stimulation of the cyclase by a directly acting agonist such as adenosine (Daly *et al.*, 1980; Daum *et al.*, 1982; Hill *et al.*, 1981) or histamine (via H_2-receptors) itself (Palacios *et al.*, 1978). It is possible that the indirect action of histamine is mediated by calcium ions (Schwabe *et al.*, 1978).

In slices of guinea-pig cerebral cortex, where there appears to be little or no H_2-component in the cyclic AMP response to histamine (Chasin *et al.*, 1973; Hill *et al.*, 1981), there is good evidence that the properties of H_1-receptors in guinea-pig brain are very similar to those in the periphery. Thus, there is a good agreement between the affinity constants of H_1-antagonists obtained from inhibition of the histamine-induced potentiation of the cyclic AMP response to adenosine and those determined from inhibition of the histamine induced contraction of guinea-pig ileum (Hill *et al.*, 1981).

Studies with [^{3}H]-mepyramine have also provided strong evidence for the presence of binding sites in brain with characteristics closely similar to those of peripheral H_1-receptors (Hill *et al.*, 1978; Chang *et al.*, 1979a). However, species differences in the affinity for [^{3}H]-mepyramine binding sites in brain homogenates occur for some H_1-antagonists but not for others (Chang *et al.*, 1979a; Hill & Young, 1980). For example, in rabbit whole brain homogenates the apparent affinities of (+)-chlorpheniramine and triprolidine for high affinity [^{3}H]-mepyramine binding sites are approximately twenty fold lower than those obtained in a corresponding membrane preparation of guinea-pig brain (Chang *et al.*, 1979a). These studies suggest that there may be species differences in the structure of the H_1-receptor. Alternatively, since there is some evidence for the presence of low-affinity promethazine-sensitive binding of [^{3}H]-mepyramine to homogenates of guinea-pig and rat brain (Hill & Young, 1980; Hadfield *et al.*, 1983), it is possible that these differences reflect variations in the proportions of high and low affinity binding sites rather than differences in H_1-receptor structure. Clearly it is important to determine whether this pattern of species variation in antagonist potency is also true of functional H_1-receptors.

Kakiuchi & Rall (1968a,b) have shown that histamine produces a large stimulation of cyclic AMP accumulation in rabbit cerebral cortical and cerebellar slices. There is some evidence to suggest that these effects of histamine can be partially antagonized by H_1-antagonists such as diphenhydramine and tripelennamine (Kakiuchi & Rall, 1968a; Palmer *et al.*, 1972; Spiker *et al.*, 1976). These observations have prompted us to examine in more detail the nature of the receptors involved in the cyclic AMP response to histamine in rabbit cerebral cortex, as a first step towards the characterization of functional H_1-receptors in the central nervous system of different species. A preliminary account of this work has been presented to the British Pharmacological Society (Al-Gadi & Hill, 1984).

Methods

Preparation of cortical slices

Rabbits (New-Zealand White, 2.5 kg) of either sex were killed by cervical dislocation, the brain removed and the cerebral cortex quickly dissected out on ice. Slices (300 × 300 μm) were cross cut with a McIlwain tissue chopper and incubated at 37°C in Krebs-Henseleit medium (75 ml per g of tissue) constantly gassed with O_2: CO_2 (95:5) in a shaking water bath for 30 min. At the end of this preliminary incubation, slices were washed with fresh Krebs medium, then suspended in Krebs medium at a concentration of 200 mg wet weight, per ml.

Drug treatment of slices and assay of cyclic AMP

Aliquots (50 μl, 10 mg wet weight) of cortical slice suspension were added to 240 μl of Krebs medium, or to Krebs medium containing antagonist drug, in 1.5 ml microfuge tubes. The tubes were gassed with O_2:CO_2 (95.5), capped and incubated for 20 min at 37°C. Agonists were added after this step in 10 μl of medium, the tubes were gassed again with O_2:CO_2 (95:5) and the incubation continued for a further 10 min. Tissue cyclic AMP was released by heating the samples on a boiling water bath for 10 min and the tissue debris then removed by centrifugation at 11,600 *g* for 2 min in a MSE Microcentaur centrifuge. Duplicate 20 or 50 μl samples were taken for cyclic AMP determination by a sensitive protein binding assay (Brown *et al.*, 1972). The tissue pellets were solubilized by heating in 1M NaOH and the protein concentration determined by the method of Lowry *et al.* (1951).

Spontaneously beating atria

Hartley strain guinea-pigs of either sex (300–500 *g*) were killed by cervical dislocation. The hearts were removed and spontaneously beating right atria were carefully dissected from the surrounding tissue and suspended in 10 ml Locke Ringer solution aerated

with oxygen (100%) in a conventional organ bath. Tissues were attached to an isotonic transducer for rate recording and measurement and were allowed to stabilize for 30 to 60 min before experimentation. Cumulative concentration-response curves were obtained to histamine, impromidine and dimaprit.

Analysis of data

Concentration-response curves for agonist stimulation of cyclic AMP accumulation were either drawn by inspection or, where the data were sufficient, fitted to a Hill equation using the Harwell library non-linear regression program VB01A. The equation fitted was:

$$\text{Stimulation of cyclic AMP production} = \frac{S_{max} \times D^n}{D^n + (EC_{50})^n}$$

where D is the agonist concentration, n is the Hill coefficient (i.e. reflects the slope of the concentration-response curve), EC_{50} is the concentration of agonist giving half-maximal stimulation and S_{max} is the maximal stimulation. Each point was weighted according to the reciprocal of the variance associated with it. Repeated trials were made with different initial parameter estimates and the final best fit values determined as those that were associated with the lowest residual sum of squares. The same non-linear minimization routine was used to fit double hyperbolae to certain of the histamine concentration-response curves. The equation fitted was:

$$\text{\% of maximal cyclic AMP production} = \frac{N \times D}{D + E_1} + \frac{(100 - N) \times D}{D + E_2}$$

where E_1 and E_2 are the EC_{50} values of histamine for the two sites and N is the percentage of the maximal response associated with the first site.

Dissociation constants, K_B, for antagonists were calculated from the parallel shift of the agonist dose-response curve using the relationship:

$$\text{Dose-ratio} = A/K_B + 1$$

where A is the concentration of antagonist and the dose-ratio is the ratio of the concentration of agonist required for a given response in the presence of antagonist to the dose of agonist required to give the same response with no antagonist present. Where appropriate the dose-ratios obtained were used to determine Schild slopes (m) by unweighted linear regression of the Schild equation (Arunlakshana & Schild, 1959):

$$\log(\text{dose-ratio} - 1) = m.\log A + \log(1/K_B)$$

In some experiments Schild slopes and antagonist dissociation constants were also determined using an iterative procedure to fit simultaneously families of concentration-response curves to a four parameter logistic equation (Waud, 1975). The equation fitted was:

$$\text{Stimulation of cyclic AMP production} = \frac{S_{max} \times D^n}{D^n + (EC_{50} \times (1 + B^m/K_B))^n}$$

where B is the antagonist concentration and the other symbols have their previous meaning.

Drugs

Mepyramine maleate and adenosine were obtained from Sigma and histamine acid phosphate from BDH. Gifts of dimaprit, 2-thiazolylethylamine (2-(2-aminoethyl)thiazole), impromidine, cimetidine and metiamide (all from Smith Kline & French), promethazine hydrochloride (May & Baker), triprolidine hydrochloride (Wellcome Foundation), ranitidine

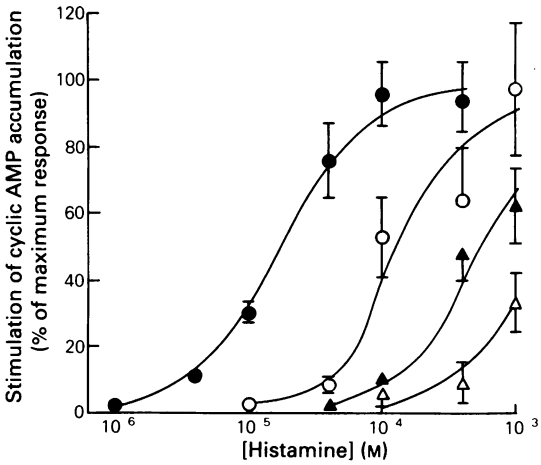

Figure 1 Inhibition by 8×10^{-6} M (○), 3.2×10^{-5} M (▲) and 8×10^{-5} M (△) cimetidine of the histamine-induced accumulation of cyclic AMP in slices of rabbit cerebral cortex; (●) control curve. To normalize responses from different slice preparations, responses are expressed as a percentage of the response to 0.1 or 0.4 mM histamine obtained in each experiment. Each point represents the combined mean of 2 to 6 experiments; vertical lines show s.e.mean. In each experiment six determinations were made at each concentration of histamine in the presence and absence of a fixed concentration of cimetidine. The curves drawn are the best-fit lines to the four parameter logistic equation (Waud, 1975) described under Methods with the following fitted parameters: maximal response 98.5 ± 4.8; EC_{50} (control curve) $1.7 \pm 0.3 \times 10^{-5}$ M; K_B $1.5 \pm 0.3 \times 10^{-6}$ M; m (Schild slope) 1.1 ± 0.1; n (Hill coefficient) 1.28 ± 0.14.

(Glaxo), tiotidine (I.C.I.), (+)- and (−)-chlorpheniramine (Schering) are gratefully acknowledged. All histamine agonists were in the form of the dihydrochloride salt, except impromidine (trihydrochloride).

Results

Characteristics of the response to histamine

Histamine (0.1 mM) elicited a large accumulation of cyclic AMP in rabbit cerebral cortical slices. The extent of the response to 0.1 mM histamine ranged from 5.5 to 74 fold above basal levels producing a mean response of 27.2 ± 2.4 fold over 42 experiments. This represented a stimulation from a mean basal level of 2.3 ± 0.2 to 53.7 ± 5.0 pmol cyclic AMP per mg protein in the presence of 0.1 mM histamine. The mean EC_{50} value obtained for histamine from 32 experiments, in which a dose-response curve was measured, was 26.3 ± 1.8 μM.

The selective H_2-receptor antagonists cimetidine (Figure 1), tiotidine, ranitidine and metiamide produced parallel shifts of the concentration-response curve to histamine to higher agonist concentrations, consistent with competitive antagonism. Where the data were adequate, Schild analysis of the dose-ratios obtained in individual experiments yielded slopes which were not significantly different from unity (cimetidine, 0.83 ± 0.13, $n = 6$; tiotidine, 0.94 ± 0.07, $n = 7$). A similar result was obtained by using an iterative procedure (Waud, 1975) to fit simultaneously families of concentration-response curves to a four parameter logistic equation as described under methods (Figure 1) on the assumption that all curves were parallel and reached the same maximum response. This analysis yielded values equivalent to the Schild slope of 1.1 ± 0.1 and 1.2 ± 0.1 for cimetidine and tiotidine respectively. The mean value obtained for the dissociation constant of cimetidine from six separate experiments, using three concentrations of antagonist, was $1.2 \pm 0.2 \times 10^{-6}$ M, in good agreement with the value obtained from inhibition of the chronotropic effect of histamine in isolated guinea-pig right atria, 7.9×10^{-7} M (Brimblecombe *et al.*, 1975). Dissociation constants for three other H_2-receptor antagonists were obtained similarly and are shown in Table 1. The basal accumulation of cyclic AMP was not altered by any of these agents. For all of the antagonists tested there was a good agreement between the dissociation constants obtained from inhibition of the histamine-

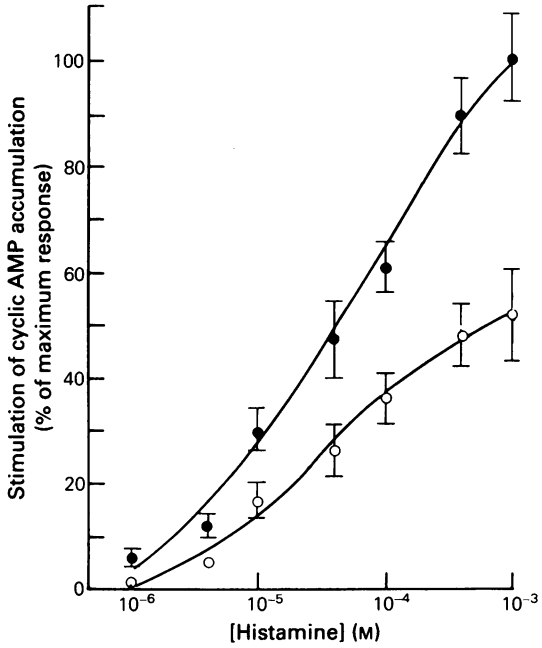

Figure 2 Inhibition by mepyramine (8×10^{-7} M; ○) of the histamine-induced accumulation of cyclic AMP in rabbit cerebral cortical slices; (●) control curve. Responses are expressed as a percentage of that produced by 1 mM histamine which was measured in each experiment. Each point represents the combined mean for six replicates in each of four separate experiments; vertical lines show s.e.means.

Table 1 Dissociation constants of H_2-receptor antagonists from inhibition of the histamine-induced accumulation of cyclic AMP in rabbit cerebral cortical slices

Antagonist	*Rabbit cerebral cortex* K_B (M)	n	*Guinea-pig atria** K_B (M)
Cimetidine	$1.2 \pm 0.2 \times 10^{-6}$	6	7.9×10^{-7} [a]
Tiotidine	$1.2 \pm 0.2 \times 10^{-8}$	7	1.5×10^{-8} [b]
Metiamide	$2.3 \pm 0.2 \times 10^{-6}$	4	9.2×10^{-7} [c]
Ranitidine	$7.2 \pm 0.8 \times 10^{-8}$	5	6.3×10^{-8} [d]

Measurements of cyclic AMP accumulation and calculation of dissociation constants were made as described under Methods.

* From inhibition of the chronotropic response of spontaneously beating guinea-pig right atria. Sources of data; [a]Brimblecombe *et al.* (1975); [b]Yellin *et al.* (1979); [c]Ganellin (1978); [d]Bradshaw *et al.* (1979).

stimulated accumulation of cyclic AMP in rabbit cerebral cortical slices and from inhibition of the histamine H_2-receptor-mediated chronotropic response in guinea-pig atria.

The H_1-receptor antagonist, mepyramine (0.8 μM), produced only a weak inhibition of the histamine stimulated cyclic AMP accumulation in slices of rabbit cerebral cortex. The combined data from four independent experiments are shown in Figure 2. The inhibition appeared to be non-competitive producing a decrease in the maximal response to histamine with little effect on the EC_{50} value.

Studies with selective H_1 and H_2-receptor agonists

Concentration-response curves for histamine, impromidine, 2-thiazolylethylamine and dimaprit are shown in Figure 3. The specific H_2-receptor agonist impromidine produced a maximum response of 31 ± 2% (EC_{50} 0.06 ± 0.01 μM, n = 4) of that produced by histamine although studies with histamine and impromidine (1 μM) in combination did not indicate that impromidine was acting as a partial agonist. Thus, in the presence of impromidine, the upper portion of the concentration-response curve for histamine (i.e. those responses larger than that produced by 1 μM impromidine alone) was not significantly different from that obtained in the absence of the selective H_2-receptor agonist (data not shown). However, the response to impromidine was antagonized competitively by the H_2-receptor antagonist, cimetidine (K_B 1.00 ± 0.01 μM, $n = 3$). In contrast, dimaprit (also H_2-selective) was without effect on the accumulation of cyclic AMP in rabbit cortical slices at concentrations up to 1 mM. Parallel studies of the chronotropic effect of impromidine, histamine and dimaprit in guinea-pig isolated right atria, however, indicated that all three agents produced the same maximal response in this tissue with EC_{50} values (μM) of 0.070 ± 0.017, 2.26 ± 0.87 and 2.46 ± 1.08 ($n = 3$, in each case) respectively. The selective H_1-receptor agonist, 2-thiazolylethylamine, produced only a weak response ($ED_{50} \sim 1$ mM) in rabbit cerebral cortical slices consistent with its potency on histamine H_2-receptors (Johnson, 1982). It is uncertain whether 2-thiazolylethylamine would have produced the same maximum response as histamine. At concentrations above 1 mM responses to agonists were often less than those at lower concentrations and in view of the non-specific effects which can occur in this very high

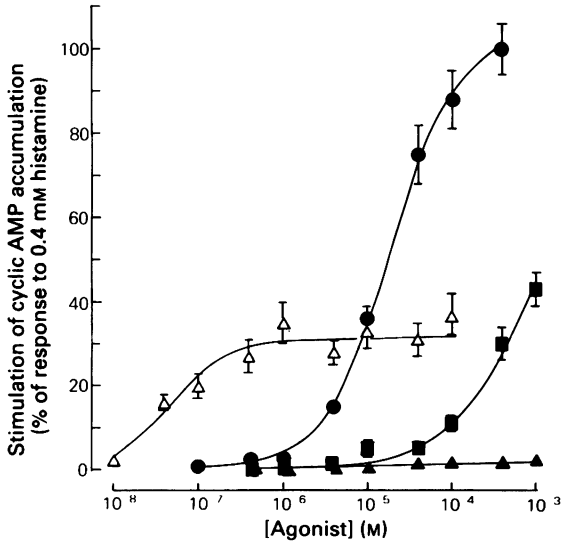

Figure 3 Concentration-response curves of the cyclic AMP accumulation induced by histamine (●), impromidine (△), 2-thiazolylethylamine (■) and dimaprit (▲) in rabbit cerebral cortical slices. To normalize responses from different slice preparations, responses are expressed as a percentage of that produced by 0.4 mM histamine which was measured in all experiments. Each point represents the combined mean from 12 (histamine) or 3 -4 (other agonists) separate experiments. Vertical lines show s.e.means. The curves drawn for histamine and impromidine are weighted best-fit lines to a Hill equation (see Methods). The curves drawn for dimaprit and 2-thiazolylethylamine were drawn by inspection.

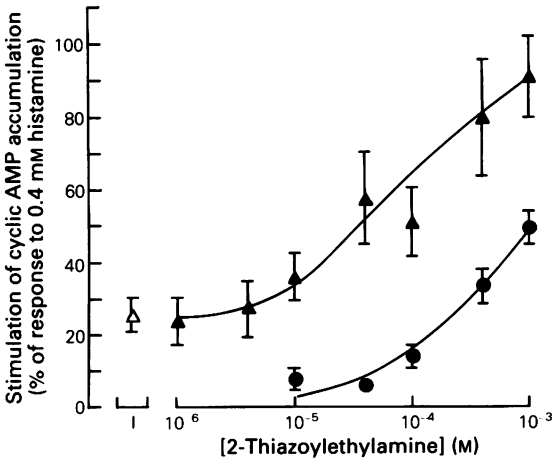

Figure 4 Concentration-response curves for 2-thiazolylethylamine obtained in the presence (▲) and absence (●) of 1 μM impromidine. The response to impromidine (I) alone is shown by the open triangle (△). To normalize responses from different slice preparations, responses are expressed as a percentage of the response to 0.4 mM histamine which was measured in each experiment. Each point represents the combined mean of three experiments; vertical lines show s.e.means. In each experiment four determinations were made at each concentration of 2-thiazolylethylamine in the presence and absence of impromidine.

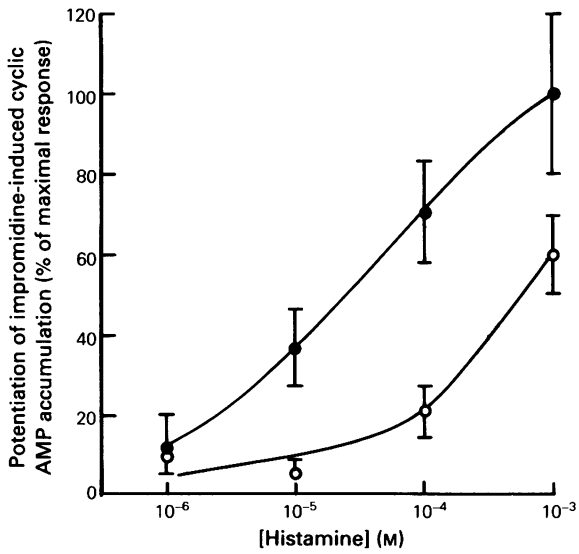

Figure 5 Concentration-response curves of the potentiation of impromidine-stimulated cyclic AMP accumulation induced by histamine in the presence (○) and absence (●) of 8×10^{-7}M mepyramine. Incubations containing 1 μM impromidine were as described under Methods. Impromidine and histamine were added simultaneously. The potentiation produced by histamine was taken to be the difference between the accumulations of cyclic AMP elicited by impromidine in the presence and absence of histamine. Responses are expressed as a percentage of that produced by 1 mM histamine. Each point represents the combined mean for eight replicates in each of four experiments; vertical lines show s.e.means.

concentration range, all response measurements were made at concentrations less than or equal to 1 mM.

To establish whether there was an indirect H_1-action on cyclic AMP generating systems in slices of rabbit cerebral cortex analogous to that found in guinea-pig hippocampus and cerebral cortex (Palacios *et al.*, 1978; Hill *et al.*, 1981), experiments were undertaken to investigate the effect of histamine and 2-thiazolylethylamine on the impromidine-induced stimulation of cyclic AMP accumulation. In the presence of a concentration of impromidine (1 μM) which just elicited a maximal response, histamine and 2-thiazolylethylalmine further stimulated the accumulation of cyclic AMP in rabbit cerebral cortical slices (Figures 4 and 5). The combined data from three independent experiments obtained with 2-thiazolylethyalamine in the presence and absence of impromidine (1 μM) are shown in Figure 4. In the presence of impromidine there was an increase in the potency of 2-thiazolylethylamine compared to that in the absence of concurrent stimulation with the selective H_2-receptor agonist. The EC_{50} value obtained for 2-thiazolylethylamine in the presence of impromidine, 44.6 ± 16 μM ($n = 5$) indicated that under these conditions the relative potency with respect to histamine in rabbit cerebral cortex (59%) was comparable with the value obtained for H_1-receptor elicited contractions of guinea-pig ileum (26%, Durant *et al.*, 1975). In the presence of impromidine, mepyramine (1 μM) appeared to antagonize competitively the potentiation produced by both histamine (Figure 5) and 2-thiazolyethylamine without affecting the basal response to impromidine alone. The dissociation constants obtained for mepyramine, assuming competitive antagonism, with either histamine or 2-thiazolylethylamine as agonist were 0.019 ± 0.008 ($n = 4$) and 0.024 ± 0.006 μM ($n = 3$) respectively. Dissociation constants for a range of other H_1-receptor antagonists were obtained similarly and are set out in Table 2. The basal level of cyclic AMP accumulation in response to 1 μM impromidine alone was not altered

Table 2 Dissociation constants of H_1-receptor antagonists determined from inhibition of the potentiation by histamine of impromidine elicited accumulation of cyclic AMP in rabbit cerebral cortical slices

Antagonist	*Rabbit cerebral cortex* K_B (M)	n	*Guinea-pig ileum** K_B (M)
Mepyramine	$1.9 \pm 0.8 \times 10^{-8}$	4	8.0×10^{-10}[a]
Promethazine	$8.0 \pm 4.0 \times 10^{-9}$	4	1.2×10^{-9}[b]
Triprolidine	$8.0 \pm 2.0 \times 10^{-8}$	3	1.0×10^{-10}[c]
(+)-Chlorpheniramine	$6.4 \pm 2.3 \times 10^{-9}$	3	7.7×10^{-10}[d]
(−)-Chlorpheniramine	$> 10^{-6}$	3	1.8×10^{-7}[d]

Measurements of cyclic AMP accumulation and calculation of affinity constants were made as described under Methods. Impromidine (1 μM) was present in every incubation.

* From inhibition of the contractile response of guinea-pig ileum segments or longitudinal muscle strips to histamine. Source of data; [a]Hill & Young (1981); [b]Marshall (1955); [c]Ison *et al.* (1973); [d]Marshall (1955) reported a value of 1.5×10^{-9} M for racemic chlorpheniramine. The dissociation constant of the active (+)-isomer has been taken as half this value. The dissociation constant of the (−)-isomer has been estimated using the potency ratio of 237 determined by Roth & Govier (1958).

Table 3 Effect of mepyramine and cimetidine on the accumulation of cyclic AMP elicited by histamine in slices of rabbit cerebral cortex in the presence and absence of adenosine

Addition	*Accumulation of cyclic AMP* (pmol per mg protein)
None	0.8 ± 0.3
Adenosine	16.5 ± 1.2
Histamine	49.5 ± 4.0
Histamine + mepyramine	34.9 ± 3.8*
Histamine + cimetidine	4.9 ± 0.8‡
Histamine + adenosine	308.7 ± 47.0
Histamine + adenosine + mepyramine	180.7 ± 18.0*
Histamine + adenosine + cimetidine	94.4 ± 11.0‡

Incubations were as described under Methods. Histamine, cimetidine and adenosine were present at a concentration of 0.1 mM and mepyramine at 1 μM. The values (mean ± s.e.mean of 8 replicate determinations) are taken from a single experiment. Essentially similar results were obtained in three other experiments.
$P < 0.05$ (*) or < 0.001 (‡) compared to data obtained in the absence of inhibitor.

by any of these agents, suggesting that at the concentrations employed there was no significant effect on H_2 – receptors. (+)-Chlorpheniramine was some 150 fold more potent than the (−)-isomer in inhibiting the histamine potentiation of the cyclic AMP response to impromidine (Table 2). This potency ratio is comparable with that found from studies of H_1-receptor-mediated responses in rabbit aorta (O'Neill & Patil, 1975) and guinea-pig ileum (Roth & Govier, 1958). However it is notable that the dissociation constants obtained for mepyramine, triprolidine, (+)-chlorpheniramine and promethazine are consistently larger than those values determined from inhibition of the H_1-mediated contractile response of guinea-pig ileal smooth muscle (Table 2) or from studies of a similar cyclic AMP response in slices of guinea-pig cerebral cortex (Hill *et al.*, 1981). In the case of triprolidine the ratio of the antagonist potencies in the two systems is particularly large (ratio of K_B values = 800; rabbit cerebral cortex: guinea-pig ileum).

Synergism between histamine & adenosine

Histamine (0.1 mM) and adenosine (0.1 mM) in combination had a much greater than additive effect on the accumulation of cyclic AMP in rabbit cerebral cortical slices (Table 3). The potentiation of the adenosine response by histamine could be partially but not completely antagonized by 0.1 mM cimetidine or 1 μM mepyramine (Table 3). The size of the cimetidine-sensitive portion of the response to a combination of histamine and adenosine varied between experiments but normally accounted for circa 60% of the final response (Table 3). It is notable, however, that the size of this component (214 pmol cyclic AMP per mg protein, in the experiment shown in Table 3) was always greater than the cimetidine-sensitive part of the response to histamine alone (44.6 pmol cyclic AMP per mg protein, Table 3). This suggests that the synergism between histamine and adenosine is dependent to a large extent on the stimulation of H_2-receptors.

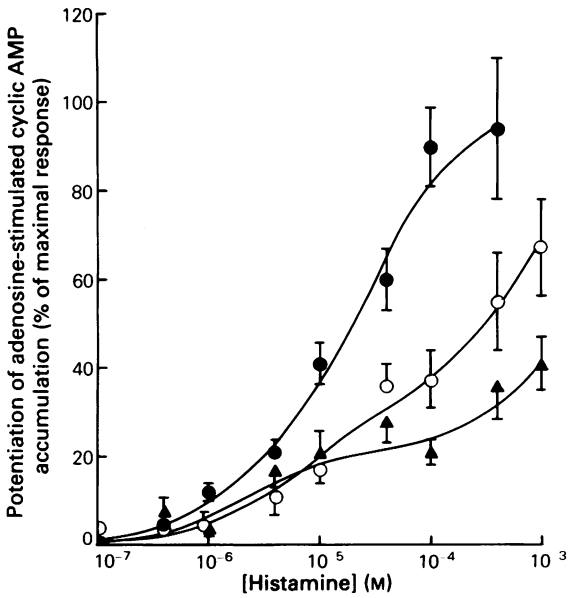

Figure 6 Inhibition by 3.2×10^{-5} M (○) and 8×10^{-5} M (▲) cimetidine of the potentiation of the adenosine-stimulated accumulation of cyclic AMP elicited by histamine in rabbit cerebral cortical slices. Responses are expressed as a percentage of the potentiation produced by 1 mM histamine. Each point represents the combined mean of 2–4 experiments; vertical lines show s.e.means. In each experiment six determinations were made at each concentration of histamine in the presence and absence of a fixed concentration of cimetidine. Adenosine (0.1 mM) was present in every incubation and added at the same time as histamine; (●) control curve.

Table 4 Analysis of concentration-response curves to histamine, obtained in the presence of cimetidine and adenosine, as double hyperbolae

Cimetidine concentration (M)	*First component* %	*First component* EC_{50} (M)	*Second component* %	*Second component* EC_{50} (M)
0	17 ± 13	$1.4 \pm 1.2 \times 10^{-6}$	83 ± 13	$2.7 \pm 1.1 \times 10^{-5}$
4×10^{-5}	34 ± 10	$7.6 \pm 4.9 \times 10^{-6}$	66 ± 10	$9.0 \pm 7.4 \times 10^{-4}$
10^{-4}	22 ± 4	$2.4 \pm 1.3 \times 10^{-6}$	78 ± 4	$3.1 \pm 1.7 \times 10^{-3}$

Values for EC_{50} and percentage of the maximum response represented by each component were obtained by fitting double hyperbolae to the experimental data in Figure 6 using the non-linear minimization routine VB01A, as described in the Methods. Adenosine (0.1 mM) was present in all incubations.

In the presence of adenosine (0.1 mM), the response to histamine (EC_{50} 11.9 ± 2.8 μM, $n = 12$) was modified in a complex fashion by cimetidine (4×10^{-5} M and 10^{-4} M) such that the response to histamine in low concentrations remained essentially unaltered while those to the amine in concentrations above 10 μM appeared to be inhibited competitively (Figure 6). To gain an impression of the likely contributions of the two components in the final response to histamine, the normalized concentration-response curves obtained in the presence and absence of different concentrations of cimetidine were fitted as double hyperbolae as described under methods. The best fit parameters for the EC_{50} values of the two sites and the percentage of the maximum response associated with each site are set out in Table 4. The EC_{50} value of the larger second component was progressively shifted to higher agonist concentrations by cimetidine. This interaction was consistent with a competitive antagonism of histamine H_2-receptors yielding a K_B value of 1.05×10^{-6} M similar to the value of 1.2×10^{-6}M obtained in the absence of adenosine (Table 1). In contrast, the first component appeared insensitive to both concentrations of cimetidine. This component was however sensitive to inhibition by H_1-receptor antagonists. In the presence of 0.1 mM cimetidine, the potentiation produced by histamine (0.1 mM) of the response to 0.1 mM adenosine (54 ± 9% of the response to histamine and adenosine alone) was further reduced to 15 ± 5% by mepyramine (1 μM; $P < 0.05$). These data suggest that histamine can potentiate the response to adenosine independently of H_2-receptor stimulation. Interestingly in guinea-pig cerebral cortex the potentiation produced by histamine of adenosine-stimulated cyclic AMP accumulation appears to be only mediated by histamine H_1-receptors (Hill *et al.*, 1981; Daum *et al.*, 1982). Furthermore, following H_2-receptor blockade with 2×10^{-5} M tiotidine (which completely abolished the response to histamine over the concentration range 10^{-6} M to 10^{-3} M, in the absence of adenosine), histamine still elicited a significant potentiation (EC_{50} 44 ± 28 μM) of the response to 0.1 mM adenosine (Figure 7). This response was antagonized by 1 μM mepyramine (Figure 7) yielding a K_B value of 0.050 ± 0.006 μM ($n = 3$) similar to that observed in studies of the potentiation of impromidine elicited accumulations of cyclic AMP (Figure 5).

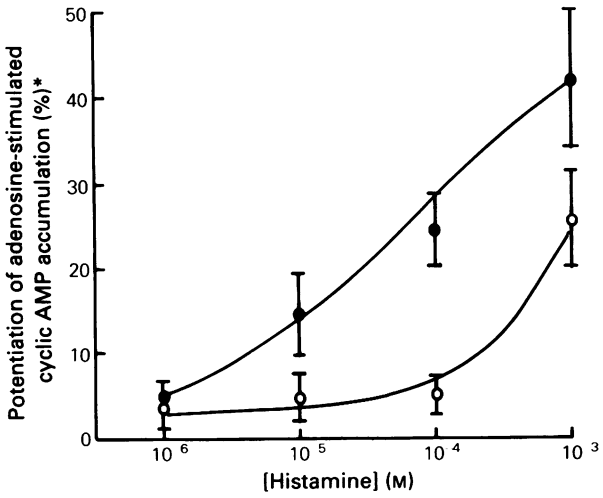

Figure 7 Concentration-response curves of the potentiation of the adenosine-stimulated cyclic AMP accumulation induced by histamine in the presence (○) and absence (●) of 1 μM mepyramine following H_2-receptor blockade with the selective H_2-antagonist, tiotidine. Adenosine (0.1 mM) and tiotidine (0.02 mM) were present in all incubations. Tiotidine was added to the incubations 20 min before the simultaneous addition of histamine and adenosine. (*)Responses are expressed as a percentage of that produced by histamine (1 mM) in the absence of tiotidine. Each point represents the combined mean of three experiments; vertical lines show s.e.means. In each experiment four determinations were made at each concentration of histamine in the presence and absence of mepyramine.

Discussion

The large stimulation of cyclic AMP accumulation

observed in slices of rabbit cerebral cortex confirms that histamine is one of the most powerful agents in stimulating cyclic AMP accumulation in the mammalian central nervous system (Daly, 1977). In many peripheral and central tissues, the effect of histamine on cyclic AMP generating systems appears to be primarily associated with histamine H_2-receptors (Johnson, 1982; and references therein). Some of our findings with selective H_2-receptor antagonists suggest that this may also be true of the cyclic AMP response to histamine in slices of rabbit cerebral cortex. For instance, all of the H_2-antagonists tested produced displacements of the concentration-response curves for histamine to higher agonist concentrations, consistent with competitive antagonism of a homogeneous population of receptors. In the case of cimetidine and tiotidine, where the data were adequate for such analysis, Schild plots gave straight lines with slopes not significantly different from unity, the value expected for a simple mass action equilibrium. Furthermore, there was excellent agreement between the equilibrium dissociation constants obtained for each antagonist from inhibition of the cyclic AMP response to histamine in rabbit cerebral cortex and from antagonism of the H_2-receptor-mediated chronotropic response of guinea-pig right atrium.

Other data, however, suggest that stimulation of H_2-receptors is not the only way in which histamine can affect cyclic AMP accumulations in rabbit cerebral cortical slices. In the presence of 0.1 mM adenosine the increase in the accumulation of cyclic AMP elicited by histamine is not completely sensitive to H_2-receptor blockade. Thus, a component of the response to low concentrations of histamine, elicited in the presence of adenosine, is insensitive to inhibition by high concentrations of H_2-antagonists such as cimetidine (0.1 mM) and tiotidine (0.02 mM). Furthermore, this cimetidine- and tiotidine-resistant component, which accounts for 20–40% of the overall response to histamine, is sensitive to inhibition by the H_1-selective antagonist, mepyramine. That such a component is not observed in the response obtained to histamine in the absence of adenosine (compare Figures 6 and 1) suggests that this component requires prior or simultaneous stimulation of adenosine receptors in order to produce its effect. A similar observation has been made in slices of guinea-pig cerebral cortex (Daly, 1977; Hill *et al.*, 1981; Daum *et al.*, 1982) where histamine appears to potentiate the cyclic AMP response to adenosine via an interaction with histamine H_1-receptors. In slices of guinea-pig hippocampus, Palacios and his colleagues (1978) have observed that a similar indirect H_1-receptor effect can be demonstrated following prior stimulation of histamine H_2-receptors. This raises the possibility that a similar interaction may occur between the two classes of receptor in rabbit cerebral cortex. Incubation of rabbit brain slices with the H_1-selective antagonist, mepyramine (0.8 μM) appeared to antagonize the response to histamine in a non-competitive manner such that there was a significant decrease in the maximal response to histamine with little effect on the EC_{50} value. This result could be interpreted as evidence for a differential effect of mepyramine on two components in the response to histamine similar to that observed in guinea-pig hippocampal slices (Palacios *et al.*, 1978). In the present case, however, the fact that mepyramine inhibited the response to histamine at all agonist concentrations (Figure 2) suggests that the EC_{50} of the indirect H_1-component is lower than that of the direct H_2-portion of the response.

Support for an interaction between two components in the final response to histamine is provided by studies with selective H_1- and H_2-receptor agonists. Impromidine produced a maximum response of only $31 \pm 2\%$ of that obtained with histamine. Studies with histamine and impromidine in combination suggest that this is due to a selective stimulation of the H_2-component of the response to histamine rather than a consequence of partial agonist activity. The H_1-selective agonist, 2-thiazolylethylamine, stimulated cyclic AMP accumulation in rabbit cerebral cortex over the concentration range 10^{-4} to 10^{-3} M. The relative potency of 2-thiazolyethylamine with respect to histamine, 2.5 (histamine = 100), is intermediate between the values obtained from studies of typical H_1- (26) and H_2-receptor (0.3) mediated responses (Durant *et al.*, 1975) and suggests that much of the response to this agonist is dependent on the stimulation of H_2-receptors. Interestingly, a similar intermediate value for the potency of 2-thiazolylethylamine (7) was observed in guinea-pig hippocampal slices where H_2- and H_1-receptors appear to be activated in a sequential manner (Palacios *et al.*, 1978). However, following maximal stimulation of the H_2-receptor component with 1 μM impromidine in rabbit cerebral cortical slices, the relative potency of 2-thiazolylethylamine was increased to 59, a value which is comparable with the value obtained for H_1-receptor-mediated contractions of guinea-pig ileum (Durant *et al.*, 1975). These data are consistent with the hypothesis that impromidine and 2-thiazolylethylamine are acting via separate mechanisms and are difficult to reconcile with a role for impromidine as a partial agonist. Furthermore, it is clear that any effect of low concentrations of 2-thiazolylethylamine on cyclic AMP levels in rabbit cerebral cortex is dependent upon the prior or conjoint stimulation of histamine H_2-receptors.

In the presence of impromidine, the potentiation produced by histamine and 2-thiazolylethylamine has many of the characteristics of an H_1-receptor mediated response. The response is inhibited by a range of H_1-antagonists of widely different structure including mepyramine and (+)-chlorpheniramine (K_B values 1.9×10^{-8} M and 6.4×10^{-9} M) which inhibit at con-

centrations very much lower than those required to inhibit histamine H_2-receptors (K_B values 2×10^{-6} M and 1.2×10^{-6} M respectively; Johnson, 1982). Furthermore, the potency ratio obtained with the stereoisomers of chlorpheniramine is particularly striking and comparable to the values obtained on H_1-receptor systems in rabbit aorta (O'Neill & Patil, 1975) and guinea-pig ileum (Roth & Govier, 1958). However, it is notable that the dissociation constants obtained for all of the H_1-antagonists tested in rabbit cerebral cortex are consistently larger than those obtained in guinea-pig ileum (Table 2) and guinea-pig cerebral cortex (Hill *et al.*, 1981). In the case of triprolidine, the ratio of the antagonist potencies in rabbit cerebral cortex and guinea-pig ileum is particularly large (800).

A striking feature of previous studies of [^{3}H]-mepyramine binding in rabbit, rat and guinea-pig tissues is that there appears to be marked species and tissue differences in the structure of the high affinity binding sites for [^{3}H]-mepyramine as indicated by the variation of the dissociation constants of certain H_1-antagonists, including mepyramine itself (Chang *et al.*, 1979a,b; Hill & Young, 1980). These studies suggest that there may be species and tissue differences in the structure of the H_1-receptor, although the differences observed may partly reflect variations in the proportions of high and low affinity binding sites (see Introduction).

The data presented in this paper from functional studies in rabbit cerebral cortex, and particularly those obtained with triprolidine, would seem to support the existence of different H_1-receptor subtypes. Interestingly, differences in the apparent antagonist affinity of mepyramine for functional H_1-receptors have also been reported in peripheral tissues such as rabbit (pA_2 8.4) and guinea-pig aorta (pA_2 9.1) (Fleisch *et al.*, 1974). Furthermore, the antagonist potencies of (+) and (−)-chlorpheniramine obtained in rabbit aorta (K_B 10^{-8} M and 10^{-6} M respectively; O'Neill & Patil, 1975) are very much lower than those obtained in guinea-pig cerebral cortex (Hill *et al.*, 1981) or ileum (Hill & Young, 1981) but similar to those obtained in rabbit cerebral cortex (Table 2). There are, however, a number of other explanations for the differences in H_1-antagonist potency observed in functional studies which deserve consideration. Thus, differences in tissue binding, diffusion and metabolism together with differences in the extent of interference with biochemical events distal to the receptor-interaction may confer tissue and species selectively in the absence of receptor heterogeneity (Angus & Black, 1980, Kenakin, 1982). Further studies, perhaps measuring ^{3}H-ligand binding and biochemical responses in the same tissue preparation, will be required to resolve this problem.

The lack of effect of the H_2-selective agonist, dimaprit, on cyclic AMP accumulation in rabbit cerebral cortex is surprising in view of the marked sensitivity of the responses of impromidine and histamine to competitive antagonism by selective H_2-receptor antagonists (Table 1). Parallel experiments with the same solutions of dimaprit in guinea-pig right atrium, however, confirm that dimaprit and histamine are equipotent in this tissue. Similar, but less striking, variations in agonist potency in different H_2-receptor systems have been reported for nordimaprit (Johnson, 1982), imidazolylpropylguanidine (Parsons *et al.*, 1975) and a number of other H_2-agonists (Johnson, 1982; and references therein). Such differences in agonist action may be indicative of differences in agonist but not antagonist selectivity of the H_2-receptors in different tissues. It should be noted, however, that other factors similar to those discussed above for the H_1-receptor may contribute to the apparent tissue selectivity of dimaprit. For example, differences in the efficiency of the mechanisms which translate receptor activation into a measurable response may confer apparent tissue selectivity if the agonist has low efficacy (Kenakin & Beek, 1980; Kenakin, 1982). Studies in guinea-pig right atrium, however, suggest that dimaprit is more efficacious than histamine (Bottomley *et al.*, 1985).

The involvement of two components in the response to histamine in rabbit cerebral cortical slices is evident from our studies. The first component appears to be mediated by histamine H_2-receptors while the second, mepyramine-sensitive, component has some of the characteristics of an H_1-receptor-mediated response and requires prior stimulation of adenosine or H_2-receptors to produce its effect. The mechanism of this indirect action is unclear. Palacios *et al.* (1978) have suggested that it may result from (a) an increased efficiency of coupling between histamine H_2- or adenosine receptors and the nucleotide regulatory unit of adenylate cyclase, (b) a decreased breakdown of cyclic AMP or (c) the release of another messenger producing one of these effects. Indeed, this later possibility involving the release of another transmitter may also explain the synergism between the responses to adenosine and H_2-receptor stimulation. Whatever the exact mechanism, such a response represents a novel means by which one neurotransmitter or modulator can amplify the response of another. This may be manifest as an increase in the size of the final response or, in a tissue where the transducing mechanisms (e.g. protein kinases) become saturated, a lowering of the concentrations necessary to elicit that final response.

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