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Histamine Receptors Coupled to [^{3}H]cAMP Accumulation in Brain: Pharmacological Characterization in a Vesicular Preparation of Guinea Pig Cortex

MAUREEN N. GANNON[1] and LINDSAY B. HOUGH[2]

Department of Pharmacology, Mount Sinai School of Medicine of the City University of New York, New York, New York 10029

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SUMMARY

The histamine-stimulated accumulation of [^{3}H]cAMP (formed by prelabeling with [^{3}H]adenine) was characterized pharmacologically in a vesicular preparation of guinea pig cortex. The H_2 antagonist cimetidine maximally blocked 80% of the response, whereas only 45% of the response could be inhibited by H_1 antagonists. A combination of H_1 and H_2 antagonists completely abolished the response. These and other findings show that both H_1 and H_2 receptors mediate the response, but 25% of the response may require simultaneous activation of both receptors. A role for adenosine as a mediator of the histamine response was investigated. Adenosine deaminase (EC 3.5.4.4., 2.5 units/ml) decreased basal [^{3}H]cAMP levels, abolished the cimetidine-resistant component of the histamine response, and reduced maximal H_1 antagonism of the histamine response to 30%. Treatment with a combination of adenosine deaminase and the calcium chelator EGTA (2 mM) appeared to eliminate the H_1 component completely. Under these latter conditions only H_2 receptors appeared to mediate the histamine response. Thus, both H_1 and H_2 receptors stimulate [^{3}H]cAMP accumulation in the vesicular preparation, but the H_1 response seems to require either concomitant adenosine or H_2 receptor stimulation and may be calcium dependent. These findings differ from those found in broken cell membrane preparations, where only H_2 receptors appear to be coupled to adenylate cyclase activation.

The pharmacological classification of the stimulation of cAMP accumulation in brain by HA suggests the importance of both H_1 and H_2 receptors (for reviews see Refs. 1 and 2). In membrane preparations, stimulation of H_2 receptors activates adenylate cyclase directly, whereas H_1 receptor stimulation has no such effect (1, 2). In more intact preparations, (e.g., brain slices) H_1 receptor activation appears to potentiate the responses elicited by directly acting agents, such as H_2 agonists (e.g., Refs. 3 and 4) or adenosine (5–8). However, a quantitative model accounting for the role of both HA receptors has been lacking.

Limitations in currently available methods have impeded the classification of receptors linked to cAMP accumulation in brain. For example, in studies with brain slices, the concentration of drugs reaching their active sites is unknown. In slices of guinea pig hippocampus (2, 3), the unusual dissociation constants for H_1 antagonists in inhibiting this HA response could reflect diffusional constraints or could imply the existence of a previously unidentified HA receptor subtype. Vesicular preparations, which are synaptosome-like fractions that have fewer barriers (9), exhibit HA responses similar to those of brain slices (9, 10), but the lack of stability of this preparation has limited its use for pharmacological characterization (11–13). A few studies have examined the effect of selected concentrations of HA agonists and/or antagonists in this preparation, but these methods did not yield enough precision for large numbers of concentrations to be studied (9–12).

We recently introduced several modifications (14) of the method of Chasin *et al.* (11) for preparing and studying vesicular preparations from brain. This improved method permitted a detailed pharmacological, quantitative characterization of HA responses linked to cAMP accumulation, which is reported in the present paper.

Experimental Procedures

Materials. Dimaprit dihydrochloride, 2-thiazolylethylamine dihydrochloride, cimetidine, and metiamide were generously donated by Smith Kline and French (Welwyn Garden City, England). [2-^{3}H] Adenine was purchased from New England Nuclear (Boston, MA); adenosine deaminase (type IV) was obtained from Sigma Chemical Co. (St. Louis, MO). All other compounds were from standard commercial sources.

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[1] Present address: Laboratory of Neuroendocrinology, Rockefeller University, New York, NY 10021.

[2] Present address: Department of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208.

ABBREVIATIONS: HA, histamine; KRB, Krebs-Ringer bicarbonate buffer; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

Preparation and incubation of vesicles. Vesicles from guinea pig (male Hartley albino, 400–450 g; Perfection Breeders, Douglassville, PA) cerebral cortex were prepared and incubated as previously described (14). In brief, following decapitation, each cerebral cortex was dissected free, chopped (1 × 1 mm) on a McIlwain tissue chopper, homogenized [four strokes in a Dounce homogenizer, (0.07–0.12 mm clearance)] in 1 ml of cold KRB (122 mM NaCl, 15 mM $NaHCO_3$, 3.0 mM KCl, 1.2 mM $MgSO_4$, 1.3 mM $CaCl_2$, 0.4 mM KH_2PO_4, 10 mM dextrose, and 10 mM sodium pyruvate, equilibrated with 95% O_2/5% CO_2, pH 7.4), and transferred into test tubes. Both pestle and vessel were rinsed with 1 ml of buffer, which was added to the homogenate sample. The homogenate was centrifuged (1000 × *g*, 15 min at 4°) and the supernatant fraction was discarded. The pellet thus obtained was resuspended in 15 volumes (original tissue, w/v) of cold KRB, with or without adenosine deaminase (2.5 units/ml). Aliquots (0.7 ml; 2–3 mg of protein) were incubated for 40 min at 37° in capped scintillation vials under 95% O_2/5% CO_2, with mixing (1 cps). [^{3}H]Adenine (1 μCi/50 μl) was added to each vial to obtain a specific activity of 2.67 Ci/mmol at 0.5 μM adenine, and this mixture was incubated for 25 min under 95% O_2/5% CO_2. Fresh buffer, sometimes containing antagonists or EGTA (0.2 ml) were added, after which the vials were flushed with 95% O_2/5% CO_2, and the incubation continued for a further 20 min with rapid shaking (3 cps). Stimulation of [^{3}H]cAMP formation was initiated with the addition of HA or other test agents (50 μl), and incubation was for 10 min under 95% O_2/5% CO_2. Under these incubation conditions (14), [^{3}H]ATP/ADP levels (cpm) had reached steady state levels, which were maintained for the duration of the assay and unaltered by the addition of buffer or test agents. The reaction was terminated by addition of 0.1 ml of trichloroacetic acid (72%), and cAMP was isolated by a procedure based on the method of Salomon et al. (15).

Agonist concentration-response curves were fitted by an iterative least squares fit to a form of the logistic function (16). Additional analyses and statistical tests were done on the PROPHET computer system.

Results

Effect of histamine in the absence of adenosine deaminase and EGTA. The H_2 antagonist cimetidine caused a concentration-dependent, surmountable shift to the right in the [^{3}H]cAMP response to HA (Fig. 1A). Increasing concentrations of cimetidine lowered the slope index of HA concentration-response curves, indicating a deviation from simple competitive antagonism (Fig. 1A). About 20% of the maximum response to HA (100 μM) was resistant to blockade by a high concentration (300 μM) of cimetidine (Fig. 1A). A cimetidine-resistant component was also observed at lower HA concentrations (1–6 μM) (FIG. 1A) and appeared to be independent of the cimetidine concentration used ($p > 0.05$, at [HA] = 1–6 μM for [cimetidine] = 1, 3, and 10 μM, by analysis of variance). This cimetidine-resistant component appeared to be dependent on HA concentration, with an estimated EC_{50} of 3 μM (see Fig. 1 and legend). When this theoretical component was subtracted from the total HA response, cimetidine caused parallel, surmountable shifts to the right of the remaining HA response (Fig. 1B). Schild analysis (17) of these data (Fig. 1B, *inset*) generated a line whose slope did not differ from unity (1.11 ± 0.09) and a pA_2 for cimetidine of 6.57 ± 0.26.

The H_1 antagonist *d*-chlorpheniramine also produced concentration-dependent changes in the [^{3}H]cAMP response to HA (Fig. 2). A low concentration of *d*-chlorpheniramine (0.01 μM) produced a small surmountable shift to the right in the HA concentration-response curve (Fig. 2). Higher concentrations caused a further decrease in the maximum HA response,

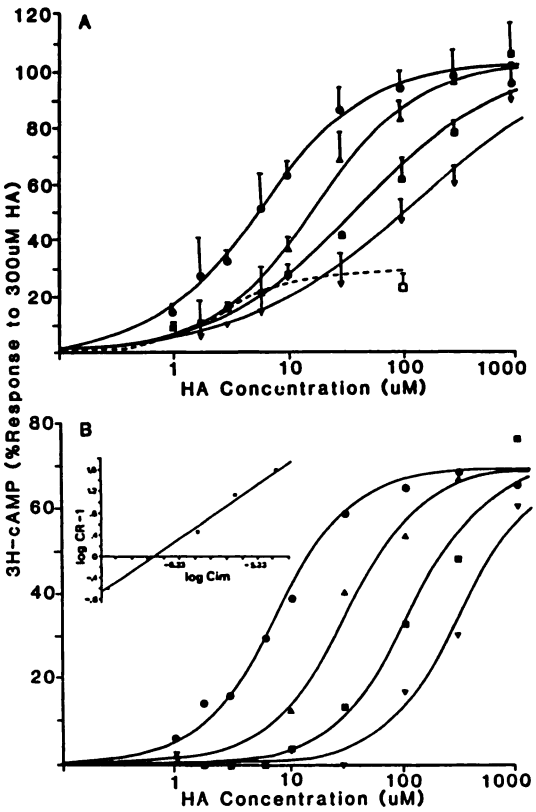

Fig. 1. Cimetidine antagonism of the [^{3}H]cAMP response to histamine, in the absence of adenosine deaminase. Vesicular preparations from two to three guinea pig cerebral cortices were prepared and incubated in KRB, as described under Experimental Procedures. Cimetidine [in μM: 0 (●) 1 (▲), 3 (■), 10 (▼), and 300 (□)] was added 20 min before incubation with HA, which was for 10 min. A. Each point for cimetidine inhibition is the mean ± SE of two to five separate experiments, each determined in triplicate. Data are normalized (100%) to 300 μM HA control values determined in each experiment (n = 10 for control). Basal and HA-stimulated [^{3}H]cAMP levels (cpm) were within the range given in Table 1. Each curve (——) represents the logistic fit to its respective cimetidine concentration, constraining all curves to a common E_{max}. A logistic fit to an apparent cimetidine-resistant component in the HA response (– – – –) was obtained by fitting to mean values obtained by pooling all cimetidine data points (1–10 μM) at 1, 1.75, 3, and 6 μM HA (which, for any given [HA], were not significantly different by analysis of variance); E_{max} = 30%, EC_{50} = 3 μM, slope index = 1.362. B. The observed increase in [^{3}H]cAMP formation was corrected for the cimetidine-resistant component described in A by subtracting the fitted function from all data points. *Inset*: Schild plot derived from the data in B.

with no additional shift in the overall HA EC_{50} (Fig. 2). A similar pattern of inhibition was seen with mepyramine (0.01–1 μM, data not shown). *l*-Chlorpheniramine (0.1 μM) had no significant effect on the HA response (Fig. 2).

Only 45% of the [^{3}H]cAMP response to HA (100 μM) was sensitive to inhibition by H_1 antagonists (Fig. 3). Maximal inhibition was attained by 1.0 μM mepyramine, 1.0 μM *d*-chlorpheniramine, or 10 μM *l*-chlorpheniramine, with no further inhibition noted when antagonist concentrations were increased by an order of magnitude (Fig. 3). Logistic fits to the data of Fig. 3 yielded IC_{50} values of 36 ± 8, 14 ± 3, and 767 ± 170 nM for mepyramine, *d*-chlorpheniramine, and *l*-chlorpheniramine, respectively. All three antagonists gave a common maximal (45.3 ± 1.1%) inhibition of the HA response.

Dimaprit, a selective H_2 agonist, also caused a concentration-dependent increase in [^{3}H]cAMP (EC_{50} = 76.5 μM; slope index

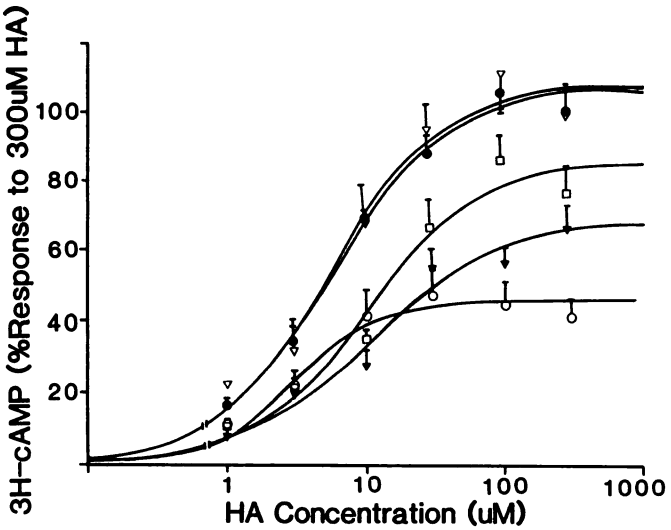

Fig. 2. Effect of chlorpheniramine on the [^{3}H]cAMP response to histamine in the absence of adenosine deaminase. Vesicular preparations were prepared from two guinea pig cortices and incubated in KRB, as described under Experimental Procedures. *d*-Chlorpheniramine [in μM: 0 (●), 0.01 (□), 0.1 (▼), and 1.0 (○)] or *l*-chlorpheniramine [0.1 μM (▽)] were added 20 min before incubation with HA, which was for 10 min. Each point represents the mean ± SE of two or four separate experiments, each determined in triplicate. Data are normalized (100%) to 300 μM HA control values determined in each experiment. Basal and HA-stimulated [^{3}H]cAMP levels (cpm) were within the range given in Table 1. Curves shown are logistic fits to the mean data points.

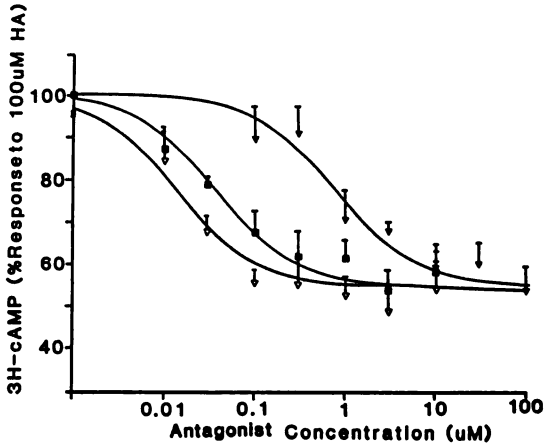

Fig. 3. Effect of H_1 antagonists on the [^{3}H]cAMP response to 100 μM histamine, in the absence of adenosine deaminase. Vesicular preparations from two to three guinea pigs were prepared and incubated in KRB, as described under Experimental Procedures. Mepyramine (■), *d*-chlorpheniramine (▽), or *l*-chlorpheniramine (▼) was added 20 min before incubation with HA, which was for 10 min. Each point represents the mean ± SE of three to seven experiments, each determined in triplicate. Data are normalized (100%) to 100 μM HA control values determined in each experiment. Basal and HA-stimulated [^{3}H]cAMP levels (cpm) were within the range given in Table 1. Curves shown were generated by fitting the data to the function:

$$\text{Response} = 100 - \frac{(100 - C)}{1 + (IC_{50}/[A])}$$

where C = fractional per cent of the HA response insensitive to H_1 antagonists, and $[A]$ = antagonist concentration $IC_{50} = [A]$ producing half-maximal effect.

= 0.502, E_{max} = 68% of the HA E_{max}, determined in paired experiments, $n = 9$). Incubation with cimetidine (3 μM) caused a shift in the concentration response to dimaprit, whereas mepyramine (0.1 μM) had no effect (data not shown).

Effect of histamine in the presence of adenosine deaminase. Adenosine deaminase altered several parameters of the HA concentration-response curve (Table 1). Although this enzyme caused an 80% reduction in basal activity, a significant decrease in maximum HA stimulation was not seen (Table 1). Adenosine deaminase also caused a small but significant shift to the right in the HA EC_{50} (Table 1). The addition of this enzyme also increased [^{3}H]ATP/ADP levels (cpm) by about 10% (data not shown).

Adenosine deaminase also caused significant changes in the sensitivity of the HA response to inhibition by H_1 antagonists and cimetidine, compared to the response measured in its absence. In the presence of adenosine deaminase, the entire response to HA (100 μM) was blocked by cimetidine (300 μM, data not shown), in contrast to results obtained in the absence of this enzyme (Fig. 1). A combination of mepyramine (3 μM) and cimetidine (300 μM) abolished the HA response in both the presence and absence of adenosine deaminase, but had no effect on basal [^{3}H]cAMP levels (data not shown).

The sensitivity of the HA response to inhibition by H_1 antagonists was reduced by adenosine deaminase (cf. Fig. 4 versus. Fig. 2). In the presence of this enzyme, the H_1-antagonists mepyramine, *d*-chlorpheniramine, and promethazine (0.1 and 1.0 μM) each caused similar concentration-dependent effects on the HA response, inhibiting the HA E_{max} by about 30% at 1 μM (Fig. 4). This fractional inhibition was significantly smaller than that seen in the absence of adenosine deaminase ($p < 0.05$, control versus adenosine deaminase for both 0.1 and 1.0 μM *d*-chlorpheniramine at 100 μM HA, $n > 2$; t test). H_1 antagonists did not cause a shift in the HA EC_{50} when adenosine deaminase was present, as they did when it was absent (cf. Fig. 4 versus Fig 2). Neither diphenhydramine nor *l*-chlorpheniramine (0.1 and 1.0 μM) inhibited the HA response in the presence of adenosine deaminase (data not shown).

Effect of histamine in the presence of adenosine deaminase and EGTA. EGTA (2 mM), in combination with adenosine deaminase, caused a decrease in basal activity similar to that seen in the presence of adenosine deaminase alone, and HA E_{max} values were reduced to 54.4% (compared to control incubations) (Table 1). HA concentration-response curves appeared monophasic in both the absence (e.g., Fig. 1) and presence (e.g., Fig. 5) of combined adenosine deaminase plus EGTA, with slope indices not significantly different from 1 (Table 1). EGTA in combination with adenosine deaminase caused a shift in the HA EC_{50} not significantly different from that seen with adenosine deaminase alone; both were significantly different from control incubations (Table 1).

The potency of H_1- but not H_2-selective agonists was decreased by the combination of EGTA and adenosine deaminase (Fig. 5). In addition to the effects of this combination on the concentration-response curve to HA (Table 1, Fig. 5), the concentration-response curve to the selective H_1 agonist 2-thiazolylethylamine was also shifted to the right (Fig. 5). The position of the concentration-response curve to dimaprit was influenced little by this treatment (Fig. 5).

In the presence of EGTA and adenosine deaminase, the H_1 antagonist mepyramine (1 μM) had no significant effect on the

TABLE 1

Effect of adenosine deaminase with or without EGTA on HA concentration-response curves

Vesicular preparations of guinea pig cerebral cortex were prepared and incubated in 15 mM KRB, with or without adenosine deaminase (2.5 units/ml) or EGTA (2 mM), as described under Experimental Procedures. HA concentration-response curves (1–1000 μM) were determined in triplicate. Shown are the means with either ± standard errors or 95% confidence limits (ranges) obtained from fitting mean HA data points from individual experiments to a logistic function. Other than the slope index, unblocked one-way analysis of variance revealed significant differences between treatment groups ($p < 0.001$ for all parameters). Significance levels given were determined by Newman-Keuls multiple range testing.

Treatment	No. of Expts.	Basal	E_{max} (total-basal)	% Increase	EC_{50}[a]	Slope index
		cpm			μM	
Control	28	4544 ±252	6150 ±423	141 ±10	5.91 4.86–7.20	1.11 0.99–1.22
2.5 Units/ml adenosine deaminase	7	943[b] ±51	5097 ±449	545[b] ±49	11.36[c] 7.97–16.18	1.05 0.84–1.27
2 mM EGTA + 2.5 units/ml adenosine deaminase	12	1062[b] ±45	2807[d] ±129	268[d] ±14	16.22[b] 12.64–20.80	0.99 0.83–1.14

[a] Geometric mean; all other values are arithmetic means.
[b] $p < 0.01$, significantly different from control.
[c] $p < 0.05$, significantly different from control.
[d] $p < 0.01$, significantly different from control and adenosine deaminase-treated groups.

HA concentration-response curve (data not shown). This observation is in contrast to the inhibition of the HA response by mepyramine in the absence of these agents (Fig. 3 and data not shown).

The H_2 antagonists cimetidine and metiamide caused a concentration-dependent shift to the right in the response to HA in the presence of EGTA and adenosine deaminase (Fig. 6 and data not shown). Schild analysis (Fig. 6) gave a slope not

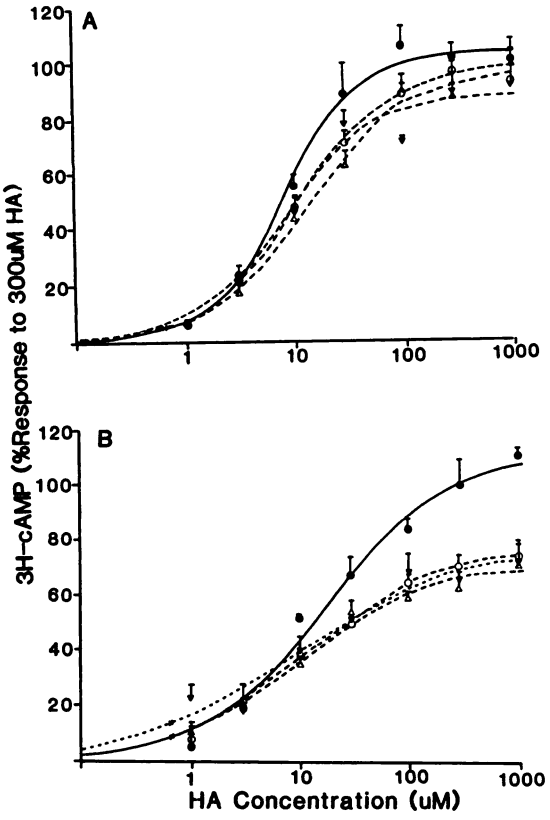

Fig. 4. Effect of H_1 antagonists on the [^{3}H]cAMP concentration response to histamine, in the presence of adenosine deaminase. Vesicular preparations were prepared from three guinea pig cortices in KRB, as described under Experimental Procedures. Incubation at 37° was conducted in the presence of adenosine deaminase (2.5 units/ml). H_1 antagonists (A, 0.1 μM; B, 1.0 μM) were added 20 min before incubation with HA, which was for 10 min. ●, HA alone; ○, HA plus mepyramine; ▼, chlorpheniramine; △, promethazine. Each point represents the mean ± SE of six data points determined from two separate experiments, each in triplicate. Data are normalized (100%) to the response obtained with 300 μM HA in each experiment. Basal and HA-stimulated [^{3}H]cAMP levels (cpm) were within the range given in Table 1. Curves shown are logistic fits to the mean data points.

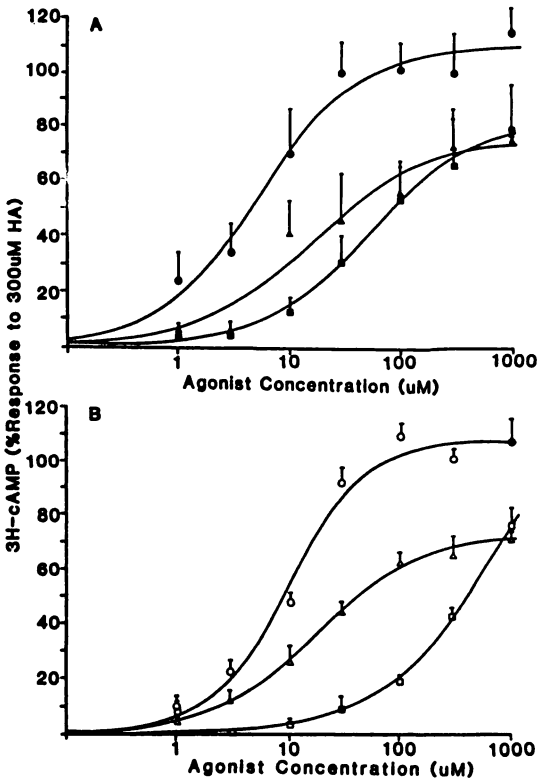

Fig. 5. Effect of EGTA and adenosine deaminase on the [^{3}H]cAMP concentration-response curves to histamine-receptor agonists. Vesicular preparations from three guinea pig cerebral cortices were prepared and incubated in 15 mM KRB, as described under Experimental Procedures. Preparations were incubated at 37° in the absence (A) or presence (B) of adenosine deaminase (2.5 units/ml). EGTA (B; 2 mM) or buffer (A) was added 20 min before incubation with HA (●, ○), dimaprit (▲, △), or 2-thiazolylethylamine (■, □), which was for 10 min. Each point represents the mean ± SE from two separate experiments, each determined in triplicate. Data are normalized (100%) to the response obtained with 300 μM HA under each incubation condition. Basal and HA-stimulated [^{3}H]cAMP levels (cpm) were within the range given in Table 1. Curves are logistic fits to the data points shown.

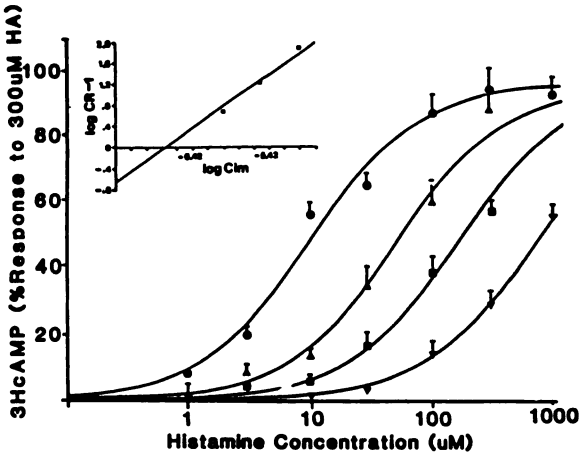

Fig. 6. Effect of cimetidine on the histamine response in the presence of EGTA and adenosine deaminase. Vesicular preparations from three guinea pig cortices were prepared and incubated, as described under Experimental Procedures. Incubation at 37° was conducted in the presence of adenosine deaminase (2.5 units/ml). EGTA (2 mM) and cimetidine [0 (●), 1 (▲), 3 (■), and 10 (▼) μM] were added 20 min before incubation with HA, for 10 min. Each antagonist curve represents the mean of six data points determined in two separate experiments, each in triplicate (control $n = 4$). Data are normalized (100%) to the control 300 μM HA response determined in each experiment. Basal and HA-stimulated [^{3}H]cAMP levels (cpm) were within the range given in Table 1. Curves shown are common slope logistic fits to the data. *Inset:* Schild plot of data.

different from unity (1.21 ± 0.20) and a derived cimetidine pA_2 of 6.74 ± 0.32 (Fig. 6, *inset*) similar to pA_2 values obtained in the absence of adenosine deaminase and EGTA (see above). This latter analysis assumes that cimetidine antagonism was surmountable, which was not demonstrated over the entire HA concentration range studied (Fig. 6). Increasing concentrations of HA above 1 mM caused a further increase in [^{3}H]cAMP levels (about 25 and 55% greater than HA E_{max} values obtained from a logistic fit to data up to and including 1 mM HA, $n = 2$), which did not appear to be influenced by the H_2 antagonist metiamide (1–10 μM, data not shown). Subtracting this component of the HA response revealed that metiamide caused parallel surmountable inhibition of the remaining HA response (not shown). Schild analysis gave a slope not different from unity (1.09 ± 0.03) and a derived metiamide pA_2 of 6.15 ± 0.12 (data not shown).

Discussion

The present study provides pharmacological evidence showing that both H_2 and H_1 receptors are linked to [^{3}H]cAMP accumulation in the guinea pig vesicular preparation. A significant H_2 component (accounting for 80% of the maximum [^{3}H]cAMP response to HA) was revealed by the inhibition of HA and dimaprit responses by the H_2 antagonist cimetidine (Fig. 1 and data not shown). The derived dissociation constant for cimetidine (0.27 μM) is similar to those obtained from other H_2-mediated processes (18) and supports the hypothesis that H_2 receptors mediate a major portion of HA-stimulated [^{3}H]cAMP accumulation observed in the present study.

An H_1 receptor involvement in the [^{3}H]cAMP response to HA is also clear. The H_1 antagonists mepyramine and *d*-chlorpheniramine both elicited concentration-dependent inhibition of part of the HA response (Figs. 2 and 3). Importantly, the *l*-isomer of chlorpheniramine was approximately 100 times less potent than its stereoisomer (Figs. 2 and 3), in agreement with the ability of these agents to inhibit [^{3}H]mepyramine binding (19) and other H_1-mediated processes (1, 8).

H_1 antagonists did not inhibit the H_2 receptor response, since mepyramine (0.1 μM) had no effect on the concentration response to dimaprit. Others have shown that the [^{3}H]cAMP formed in response to the selective H_2 agonist 4-methyl-HA (100 μM) is not antagonized by the H_1 antagonist tripelennamine (0.3–10 μM) (10). Higher concentrations of H_1 antagonists (>10 μM) are also required to inhibit the cAMP response to H_2 receptor stimulation in brain slices (20).

A fraction of the response to H_1 receptor activation appears to require simultaneous stimulation of H_2 receptors for expression (Fig. 7). In contrast to the large proportion of H_2 receptor involvement in the response to HA (80%), only 45% of the overall response appeared to be mediated by H_1 receptor stimulation (Fig. 3). The remaining 55% was not blocked by H_1 antagonists at concentrations up to 100 μM, and was probably mediated through H_2 receptor stimulation, since a combination of H_1 and H_2 receptor antagonists completely blocked the HA response. Since 80% of the HA response was blocked by an H_2 antagonist and 45% by H_1 antagonists, these results suggest that 25% of the HA response requires simultaneous activation of H_1 and H_2 receptors. Consistent with these observations, in a similar preparation of guinea pig cerebral cortex, Psychoyos (10) demonstrated that at least 70% of the [^{3}H]cAMP response to 2-methyl-HA (100 μM) [a selective H_1 agonist which would be predicted to activate both H_1 and H_2 receptors at this concentration (21)] was inhibited by the H_2 antagonist metiamide (10 μM) and up to 80% was inhibited by tripelennamine (0.1–3 μM), suggesting that some fraction of the response to 2-methyl-HA required activation of both H_1 and H_2 receptors.

Our results are consistent with the hypothesis that endogenous adenosine potentiates the [^{3}H]cAMP response to H_1 re-

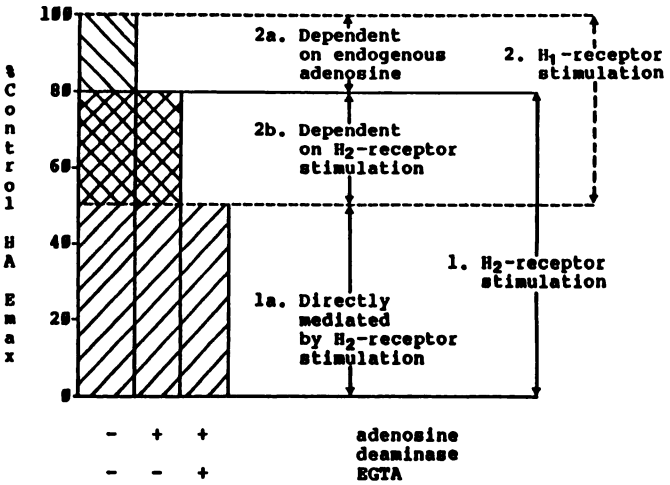

Fig. 7. Relative distribution of putative histamine receptors mediating histamine-stimulated [^{3}H]cAMP accumulation in the vesicular preparation. Schematic representation of the proportional distribution of putative HA receptors mediating the [^{3}H]cAMP response to HA, in the absence or presence of adenosine deaminase with or without EGTA. The responses to HA are expressed as a fraction of the maximum HA response obtained in the absence of adenosine deaminase and EGTA (from Table 1). Putative H_2 receptor or H_1 receptor involvement in the HA response was inferred from the inhibition obtained in the presence of H_2 (Figs. 1 and 6, and data not shown) or H_1 antagonists (Figs. 2, 3, and 4, and data not shown), under the different incubation conditions. See the text for further details.

ceptor stimulation. Thus, adenosine deaminase caused a large decrease in [^{3}H]cAMP basal activity (Table 1), suggesting that extracellular adenosine concentrations were sufficient to activate adenosine receptors coupled to [^{3}H]cAMP accumulation in this and other studies of vesicular (12) and slice (8) preparations of brain. This reduction in basal activity was accompanied by a reduction in the sensitivity of the HA response to inhibition by H_1 antagonists (cf. Fig. 2 versus Fig. 4), with no significant change in the maximum response to HA (Table 1) or dimaprit (100 μM). In a similar study of the vesicular preparation, adenosine deaminase caused a significant (60%) decrease in the [^{3}H]cAMP response to HA (100 μM) (12), also associated with a decreased sensitivity to inhibition by H_1 antagonists.

Non-receptor-mediated actions of adenosine (and/or adenosine deaminase) might also contribute to the current results. Since we observed an adenosine deaminase-induced increase in [^{3}H]ATP/ADP levels, and others have shown specific activity changes in the [^{3}H]cAMP response to noradrenaline (11), the role of possible nucleotide pools in adenosine modulation of neurohormone-responsive cAMP-generating systems requires further clarification. However, adenosine receptor antagonists mimic the effects of adenosine deaminase on the magnitude of the [^{3}H]cAMP response to H_1 receptor stimulation (9), supporting the current interpretation that adenosine deaminase effects occur by removal of adenosine from its extracellular receptor.

In guinea pig cortical brain slices, addition of exogenous adenosine increased the maximum response to HA agonists (6), which was associated with increased inhibition by H_1 antagonists. In rabbit cortical slices (4), a cimetidine-insensitive component in the HA response was observed in the presence, but not absence, of adenosine (100 μM), a finding paralleled by the current study. Thus, both endogenous and exogenous adenosine appear to potentiate H_1 receptor-mediated cAMP accumulation in vesicular or slice preparations of brain.

The effects of H_2 antagonists in the presence of adenosine deaminase also support the hypothesis that H_1 receptor responses require activation of H_2 receptors. In the presence (but not the absence) of adenosine deaminase, the entire response to HA was inhibited by cimetidine; the H_1 component, although still evident, was reduced. Interestingly, in guinea pig hippocampal (10) or rabbit cortical (4), but not guinea pig cortical (5–8), slices, the cAMP response to HA is also entirely blocked by H_2 antagonists and partially inhibited by H_1 antagonists.

It seems likely that H_1-mediated cAMP accumulation in the vesicular preparation, as in brain slices (22, 23), is at least partially calcium dependent. In the presence of adenosine deaminase and EGTA, mepyramine had no effect on the HA concentration response. In addition, the concentration-response curves to the mixed receptor agonists 2-thiazolylethylamine and HA, but not to the selective H_2 agonist dimaprit, were shifted to the right, generating EC_{50} values similar to those obtained in other H_2-mediated processes (21). These observations suggest that a combination of EGTA and adenosine deaminase eliminated the H_1 component in the HA response. Adenosine deaminase and EGTA also decreased the maximum response to HA (Table 1) but not to dimaprit (100 μM), suggesting a selective removal of the H_1-mediated component (Fig. 7).

Evidence from brain slice studies suggests that the H_1 response may not have an absolute requirement for calcium. Incubation of cortical brain slices in calcium-free buffer did not reduce the cAMP response to a combination of dimaprit and 2-thiazolylethylamine to that observed with dimaprit alone (23), a predicted effect if the response to 2-thiazolylethylamine is through a calcium-dependent H_2 receptor. In a similar study (22), approximately 50% of HA-stimulated (100 μM) [^{14}C] cAMP accumulation was blocked by the H_1 antagonist brompheniramine (100 μM) in the presence of EGTA (2 mM), while a combination of EGTA and the adenosine receptor antagonist theophylline (200 μM) abolished this H_1 antagonist sensitivity. This latter study suggests that H_1 receptor stimulation may increase the cAMP response to endogenous adenosine in the absence of calcium, particularly since EGTA may increase basal activity due to increased adenosine release (24, 25). This possibility was not investigated in the current study, since EGTA (2 mM) alone caused a large increase in basal activity, which appeared to mask the response to HA.

Only H_2 receptors appeared to be linked to [^{3}H]cAMP accumulation in the presence of EGTA and adenosine deaminase. Under these conditions, the remaining HA response was antagonized by the H_2 antagonists cimetidine and metiamide, with derived pA_2 values (6.74 and 6.15, respectively) consistent with H_2 receptor blockade (18). Interestingly, the observation that high (>1 mM) concentrations of HA cause a further increase in [^{3}H]cAMP levels has also been observed in membrane preparations (26); this response is insensitive to either H_1 or H_2 receptor antagonists.[3] Although this response appeared to be insensitive to H_2 antagonists in the current study, the possible involvement of an H_1 receptor was not excluded (i.e., EGTA caused a shift in the EC_{50} of HA at H_1 receptors to >1 mM).

The pattern of inhibition of the HA response seen in the presence of H_1 and H_2 receptor antagonists is not that expected if H_1 and H_2 receptors were independently coupled to [^{3}H] cAMP accumulation in the vesicular preparation (27, 28). According to such a model, H_1 and H_2 antagonists would inhibit only that portion of the HA response attributable to direct stimulation of H_1 and H_2 receptors, respectively, e.g., 45% sensitive to inhibition by H_1 antagonists, 55% sensitive to inhibition by H_2 antagonists. In contrast, in the present study, the degree of inhibition of the HA response by H_1 and H_2 antagonists alone was 45% (Figs. 2 and 3) and 80% (Fig. 1), respectively. Since the combined inhibition cannot be greater than 100%, these results indicate that 25% of the HA response (45% + 80% = 125%) is attributable to the simultaneous activation of H_1 and H_2 receptors. Furthermore, adenosine deaminase reduced the HA response to one where the H_1 component was entirely dependent on simultaneous H_2 receptor stimulation (Fig. 7). A dependence of H_1 receptor-mediated cAMP accumulation on simultaneous H_2 receptor activation was also observed in brain slice studies (4, 10). These observations do not agree with those predicted for an independent two-site model.

The shifts observed in the HA concentration-response curve after either H_1 or H_2 antagonists are also inconsistent with an independent two-site model. In this model, only one class of antagonists should shift the response to low concentrations of HA (i.e., the response to stimulation of the receptor subtype with the lower EC_{50}). Higher concentrations of an antagonist

[3] S. Maayani, personal communication.

would shift the upper part of the HA concentration response, while having no effect on that part of the curve insensitive to the antagonist. In the present study, both H_1 (Fig. 2) and H_2 antagonists (Fig. 1) shifted the entire HA response to the right prior to a selective effect on the upper part of the response.

Although an independent two-site model cannot account for the present data, part of the H_1 receptor-mediated response to HA appeared independent of simultaneous H_2 receptor activation, as evidenced by a cimetidine-insensitive component (Fig. 1). This component appeared dependent on endogenous adenosine, as discussed above. The pharmacological characteristics of the HA response can thus be accounted for by a metactoid sensitization model (29, 30), in which H_2 and adenosine receptor stimulation directly increases [^{3}H]cAMP accumulation, and H_1 receptor stimulation indirectly potentiates the response to either of these direct stimuli (Fig. 7). Simulation of such a model system and fitting the present results to such a model have been performed (30).

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Send reprint requests to: Dr. Lindsay B. Hough, Department of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208.