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Kinetic Studies on the Mechanism by which Histamine H_1 Receptors Potentiate Cyclic AMP Accumulation in Guinea Pig Cerebral Cortical Slices

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SUMMARY

Histamine, acting via H_1 receptors, augments adenosine-induced cAMP accumulation in slices of guinea pig cerebral cortex by an indirect mechanism that appears to involve an intracellular messenger(s). To better characterize this process, the time course of this augmentation was examined in slices prelabeled with [^{3}H] adenine. When histamine (1 mM) was added after the cAMP level had reached steady state with adenosine (0.1 mM), the cAMP level rose to a new steady level within 10 min ($t_{½}$, 2–3 min). There was no measurable delay in this response, indicating rapid activation of the augmentation after receptor occupation. Studies using the H_1 receptor antagonist mepyramine indicated that the continued presence of the histamine stimulus was required to maintain the augmentation. Addition of mepyramine (10 μM) between 1 and 14 min after histamine caused cAMP levels to fall to a level similar to that obtained previously with adenosine alone, but with a delay of 2–3 min. This gives an upper estimate of the lifetime of any intracellular messenger involved in the augmentation process. To determine whether histamine acts by stimulating synthesis of cAMP or by inhibiting its breakdown, the fall in tissue cAMP content was studied after rapid removal of the adenosine stimulus by addition of adenosine deaminase. The initial fall was significantly faster in slices incubated with 0.1 mM adenosine plus 1 mM histamine than in slices with 0.1 mM adenosine alone, indicating increased synthesis and breakdown of cAMP in the presence of histamine. However, the higher breakdown rate probably reflects stimulation of the degradation process by the higher initial level of cAMP with histamine because, at equivalent levels, cAMP content fell at similar rates in both conditions. This was confirmed in other experiments in which similar steady state cAMP levels were achieved with and without histamine by appropriate choice of adenosine concentrations. It is therefore concluded that the direct effect of histamine is primarily to potentiate cAMP synthesis.

Neurotransmitters or neuromodulators can modify cAMP levels in brain slices by two mechanisms: (a) by stimulating receptors directly coupled to the nucleotide regulatory components (N_s or N_i) of adenylate cyclase or (b) by stimulating receptors that appear to be indirectly linked to the enzymes involved in cAMP turnover and action of which is to potentiate cAMP accumulation induced by a directly acting transmitter (1–8). By this latter mechanism, histamine H_1, α_1-adrenoceptor, and γ-aminobutyric $acid_B$ receptor agonists can potentiate the cAMP responses to receptor agonists that stimulate adenylate cyclase directly (e.g., adenosine A_2, histamine H_2, and β adrenergic). It remains to be established whether the augmentation of cAMP levels is exerted via an action on adenylate cyclase (i.e., on N protein or catalytic unit) or by inhibition of the enzyme which breaks down cAMP, PDE.

Little is known about the intracellular mechanisms by which neurotransmitters indirectly potentiate cAMP accumulation. However, the production of another second messenger seems to be involved because the effect is lost in membrane preparations (9–12). Both calcium ions and the products of inositol phospholipid breakdown (the inositol phosphates and diacylglycerol) have been implicated as mediators of this augmentation response (8, 13–15).

In the present study, we have attempted to determine whether indirectly acting H_1 receptor occupation augments the adenosine A_2 receptor-mediated cAMP accumulation by potentiating cAMP synthesis or by inhibiting its breakdown. This was done by investigating the effect of H_1 receptor stimulation on the rates of cAMP synthesis and breakdown at steady state in guinea pig cerebral cortical slices. The kinetics of the adenosine-mediated response and of its potentiation by H_1 receptor stimulation are, however, poorly characterized. We have therefore undertaken a detailed analysis of the time course of these responses, as an essential part of this study.

Methods

Measurement of [^{3}H]cAMP accumulation. cAMP accumulation was determined using a modification of the [^{3}H]adenine prelabeling

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ABBREVIATIONS: PDE, phosphodiesterase, ADA, adenosine deaminase; 2CA, 2-chloroadenosine; 8PT, 8-phenyltheophylline.

technique (16). Slices (300 × 300 μm) of cerebral cortex from Hartley strain guinea pigs were labeled with [^{3}H]adenine as described previously (17). After labeling, slices were washed three times with 50 ml of Krebs-Henseleit solution at 37° and finally allowed to settle under gravity. For time course studies, slices (1-ml packed volume) were transferred to a 20-ml glass vial containing Krebs-Henseleit solution (8 ml) and, where appropriate, the H_2 receptor antagonist tiotidine (30 μM) and ADA (1.2 units/ml). Tiotidine and ADA were present to remove the direct actions of histamine (acting via H_2 receptors) and endogenous adenosine, respectively. The slice suspension was stirred magnetically at a rate sufficient to buoy up the slices and was gassed with O_2/CO_2 (95:5) via a glass Pasteur pipette positioned to direct a jet of gas on to the surface. A pH of 7.4–7.5 was well maintained using this method. At various times before and after agonist addition, 300-μl aliquots of the slice suspension were taken for analysis of cAMP.

In experiments using tetrodotoxin, incubations were performed in flat-bottomed insert vials. In these experiments, 50-μl portions of gravity-packed slices were added to 240 μl of Krebs medium containing tiotidine (30 μM) and tetrodotoxin (5 μM) where appropriate. Tubes were gassed with O_2/CO_2 (95:5), capped, and incubated for 15 min at 37° in a shaking water bath. Agonist was added after this step in 10 μl of medium and the tubes were gassed again and incubated for an additional 10 min.

Incubations or aliquots from them (300 μl) were stopped and cAMP was extracted by mixing with 200 μl of ice-cold 1 M HCl. Samples were vortexed and left on ice for at least 15 min before being diluted with 750 μl of distilled water. Slices were precipitated by brief centrifugation at 1000 × *g*. A 100-μl sample of the aqueous supernatant was taken and the total radioactivity was determined by liquid scintillation counting. These "totals" were used to correct for variations in the number of slices in each sample (±10–20% of the mean). A 1-ml sample of the supernatant was taken for analysis of [^{3}H]cAMP by column chromatography. The recovery of cAMP from the columns was corrected for by "spiking" the samples with 100 μl of [^{14}C]cAMP (0.001 μCi, 0.24 μM). Recovery was routinely approximately 80%.

[^{3}H]cAMP was isolated by sequential Dowex-alumina chromatography as described previously (17). [^{3}H] and [^{14}C]cAMP in the final eluant were determined by liquid scintillation counting on a dual label program.

In time-course studies, cAMP was measured at single time points. Each experiment was repeated 3–7 times; *n* in the text refers to the number of separate experiments, unless otherwise stated. All values for the extent of stimulation of cAMP accumulation by agonists are expressed as fold over basal (mean ± standard error).

Chemicals. Dowex 50W, H^+ form (200–400 mesh), 2CA, adenosine, ADA (type VI), neutral alumina (type WN-3), imidazole, mepyramine maleate, histamine dihydrochloride, and 8PT were purchased from Sigma Chemical Co., Poole, Dorset, England. 8-^{3}H-adenine (specific activity, 26 Ci/mmol) was obtained from Amersham International, Aylesbury, Buckinghamshire, England, and [8-^{14}C]cAMP (specific activity, 42.4 mCi/mmol) from Dupont-NEN Sterenage, Hertfordshire, England. Tiotidine was a gift from ICI Macclesfield, Chesire, England.

Results

Time course studies. Addition of adenosine (0.1 mM) to guinea pig cerebral cortical slices resulted in a rapid accumulation of [^{3}H]cAMP (Fig. 1). A steady state level (47.2- ± 9.6-fold over basal, $n = 6$, three experiments like Fig. 1 plus three others) was achieved within 10 min (half time, $t_{½} = 1.5 \pm 0.1$ min, $n = 6$). Addition of ADA (1.2 units/ml) during the steady state caused a rapid return of cAMP levels to basal with a similar $t_{½}$ (1.5 ± 0.1 min, $n = 6$) (Fig. 1). Addition of the same dose of adenosine (0.1 mM) simultaneously with 1 mM histamine also resulted in a rapid increase in the level of cAMP. A concentration of 1 mM histamine was chosen to produce a maximal augmentation of cAMP accumulation. The E_{50} of

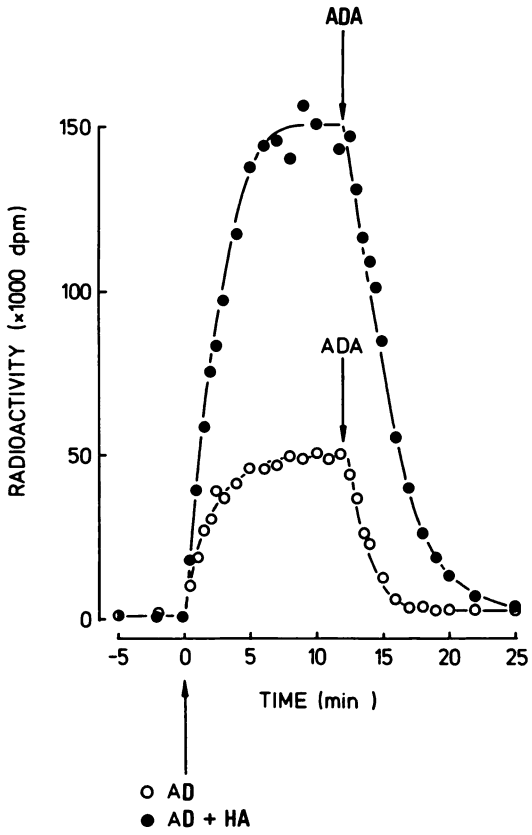

Fig. 1. Time course of the accumulation of [^{3}H]cAMP in guinea pig cerebral cortical slices after addition of 0.1 mM adenosine (*AD*) or 0.1 mM adenosine plus 1 mM histamine (*HA*) at 0 min. ADA (1.2 units/ml) was added 12 min after the agonists. The experiment was performed in the presence of 30 μM tiotidine to prevent the actions of histamine exerted via H_2 receptors. Data were obtained in a single experiment. Similar results were obtained in two other experiments.

histamine for this response was 7.53 ± 1.67 μM and this was competitively inhibited by the H_1 antagonist mepyramine (dissociation constant $K_D = 0.6$ nM) (3). An apparent steady state level of cAMP was again achieved, but at a much greater level than that with adenosine alone (132.6- ± 21.9-fold over basal, $n = 5$). The half time to steady state was slightly increased (2.5 ± 0.1 min, $n = 5$).

The responses both to histamine alone and to histamine plus adenosine were totally abolished by addition of ADA, i.e., by removal of the adenosine stimulus (Fig. 1). This indicated that, without the presence of the direct adenosine stimulus, there was no response to H_1 receptor activation. The fact that cAMP levels returned to basal after addition of ADA ($t_{½}$, 3.0 ± 0.4 min, $n = 5$) also revealed that 30 μM tiotidine was effective in abolishing the direct H_2 receptor-mediated cAMP response to histamine and justified its use in subsequent experiments. The H_1 receptor-mediated response is likely to be mediated by an intracellular rather than an intercellular mediator, because tetrodotoxin (5 μM), which prevents nerve conduction, was without effect on the response in three experiments (data not shown).

In order to confirm that ADA was only removing adenosine and not affecting cAMP levels directly, the effect of this enzyme on the response to a combination of adenosine and 2CA was investigated (Fig. 2). 2CA is a stable adenosine analogue that does not appear to be incorporated into cells (18–20). A sub-

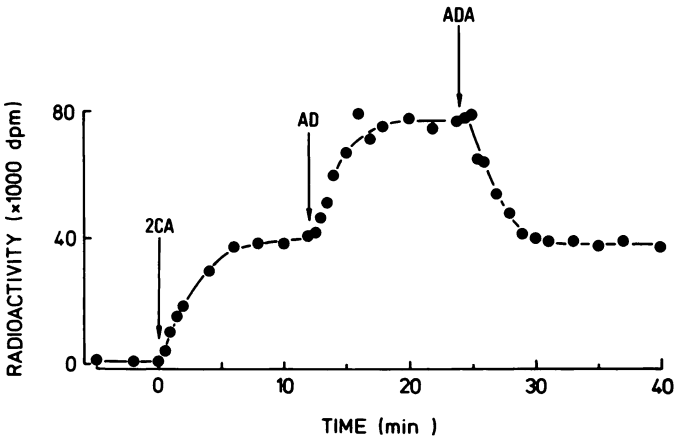

Fig. 2. Time course of the accumulation of [^{3}H]cAMP in guinea pig cerebral cortical slices after addition of 2CA (30 μM) and adenosine (0.1 mM) (*AD*). ADA (1.2 units/ml) was added 24 min after 2CA. Data shown were obtained in a single experiment but are similar to results obtained in two other experiments.

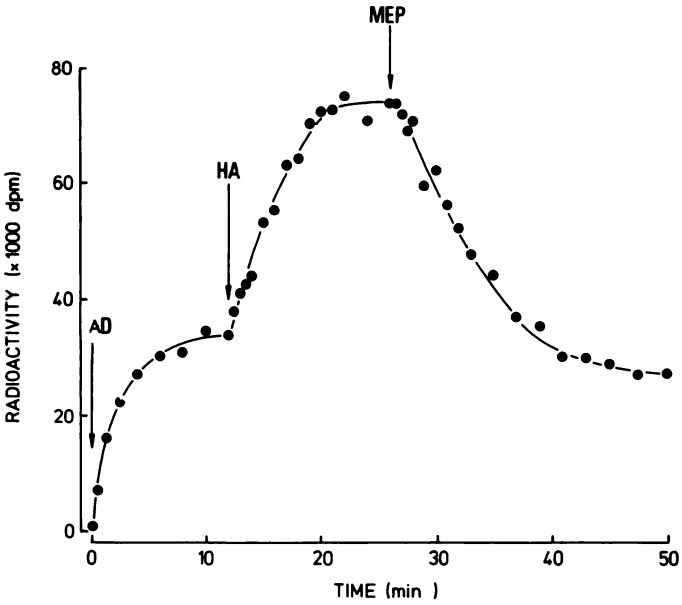

Fig. 3. Time course of the accumulation of [^{3}H]cAMP in guinea pig cerebral cortical slices after addition of adenosine (0.1 mM) (*AD*) and histamine (1 mM) (*HA*). Mepyramine (10 μM) (MEP) was added 24 min after adenosine. Experiments were performed in the presence of tiotidine (30 μM) to prevent effects of histamine mediated via H_2 receptors. Data were obtained in a single experiment. Similar results were obtained on two other occasions.

maximal dose of 2CA (30 μM) was added and a steady level of cAMP was achieved within 10 min (Fig. 2). After addition of 0.1 mM adenosine during this steady state, cAMP levels rose to form a new steady state, again within 10 min. Subsequent addition of ADA (1.2 units/ml) caused cAMP levels to fall to a level equivalent to that obtained with 2CA alone (Fig. 2), therefore suggesting that ADA reverses the cAMP response to adenosine by removing adenosine and not by altering cAMP turnover in some other way.

It was also notable from the data in Fig. 2 that the response to 2CA remained constant throughout the experiment. This suggested that the adenosine receptors did not desensitize on prolonged stimulation. This was confirmed in subsequent experiments, which showed no measurable reduction in cAMP accumulation in response to 0.1 mM adenosine over 45 min (n = 5; data not shown). The fact that this response was well maintained enabled us to investigate the time course of the augmentation of the adenosine response produced by H_1 receptor stimulation separately.

When histamine (1 mM) was added 12 min after adenosine (0.1 mM) (i.e., during the adenosine steady state) the [^{3}H] cAMP level rose to a new steady level (increasing from 53.8- ± 5.4-fold to 139.7- ± 18.9-fold over basal, n = 7) within 10 min (Fig. 3). There appeared to be no delay in the onset of the augmentation. The initial rate of cAMP accumulation after histamine addition was similar to that after adenosine (Fig. 3), but the $t_{1/2}$ was longer (3.3 ± 0.1 min, n = 7). Similar results were obtained when the stable adenosine analogue 2CA was used instead of adenosine. A $t_{1/2}$ of 2.6 ± 0.2 min (n = 4) was obtained for the augmentation of the 2CA cAMP response by histamine.

Effect of mepyramine. When the histamine H_1 stimulus was removed by addition of the H_1 receptor antagonist mepyramine during the histamine plus adenosine steady state, the cAMP level fell to a level similar to that obtained with adenosine alone (Fig. 3), indicating that continued presence of histamine was required to maintain augmentation. This finding that mepyramine could reverse the effect of histamine suggested that, by following the decay of cAMP levels after mepyramine addition in slices incubated with adenosine plus histamine, it might be possible to assess the lifetime of any "second messengers" involved in the indirect histamine augmentation response.

The results of such a study are shown in Fig. 4. In preliminary experiments there appeared to be some delay between mepyramine addition and the start of the fall in tissue cAMP content. It was not certain whether this represented the time taken for mepyramine to displace histamine from the receptors or whether it indicated a delay in switching off the messenger system. To minimize the time taken for an antagonist to displace an agonist from receptors, it is clearly desirable to maximize the dose of antagonist and in most experiments the concentration of mepyramine was 10 μM (although in a few experiments using 1 μM mepyramine similar results were obtained). A higher dose was avoided because of possible nonspecific effects (21). Mepyramine at 10 μM was without effect on the cAMP response to adenosine (Fig. 4A) and completely prevented the H_1 receptor-mediated augmentation when added together with histamine during an adenosine steady state (Fig. 4A).

Fig. 4, B–D show the effect of adding mepyramine 1, 3, and 14 min after addition of 1 mM histamine to slices in which a steady level of cAMP had been established with 0.1 mM adenosine. In all cases, cAMP levels returned to the level existing before histamine was added but there appeared to be a delay of 2–3 min after mepyramine addition before cAMP levels started to fall. These results provided further evidence that the continued presence of histamine was required to maintain the augmentation.

Time-dependent loss of cAMP responses. It was notable in some experiments that, when the response to histamine plus adenosine was followed over an extended period, the cAMP levels appeared to decrease with time (e.g., control condition in Fig. 4D). This is unlikely to be due to a change in the specific activity of the [^{3}H]cAMP, for when total cAMP levels were

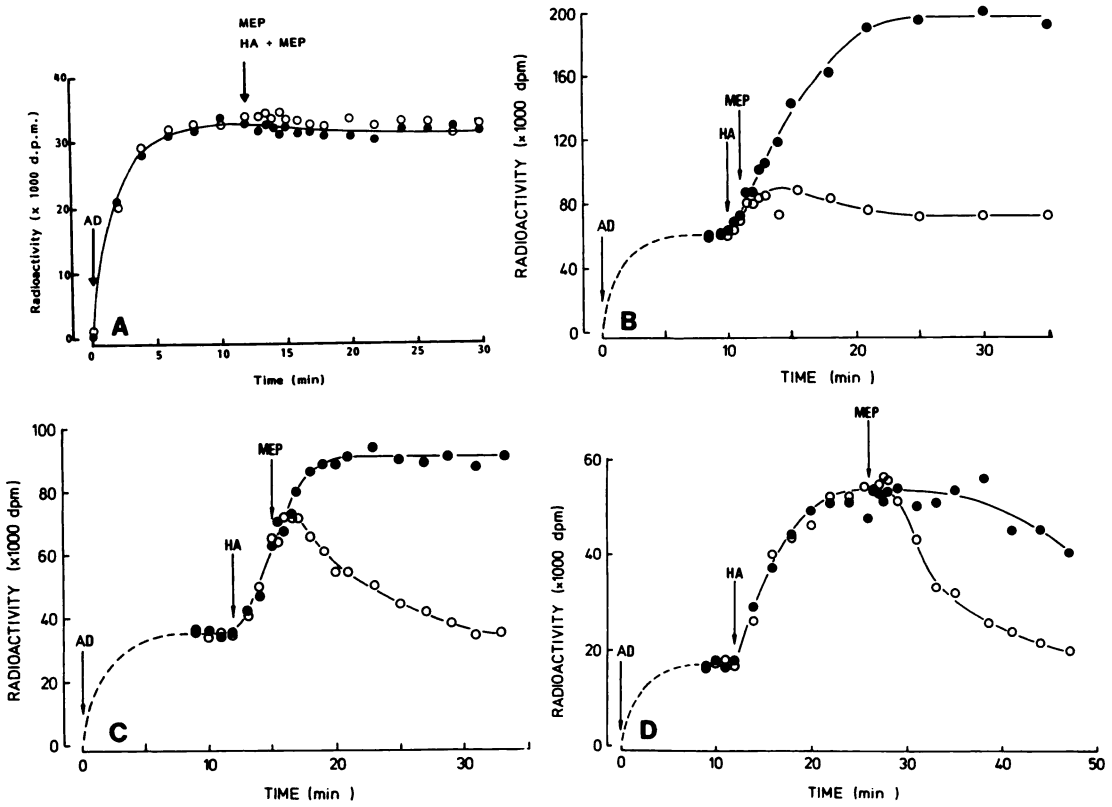

Fig. 4. Effect of mepyramine (10 μM) on the histamine H_1 receptor-mediated augmentation of the cAMP response to adenosine (*AD*) in guinea pig cerebral cortical slices. Adenosine (0.1 mM) was added at 0 min. Histamine (1 mM) (*HA*) was added after 12 min, followed by mepyramine (10 μM) (*MEP*) 0 (A), 1 (B), 3 (C), or 14 (D) min later. In A, mepyramine (●) or mepyramine plus histamine (○) were added 12 min after adenosine. In B, C, and D, ○ represents data for the mepyramine-treated slices and ● represents data for control slices in which Krebs-Henseleit medium was added to the slice suspension instead of mepyramine. *Dotted lines* represent the approximate time course of [^{3}H]cAMP accumulation after adenosine addition, based on the data of Fig. 1. Each panel represents data obtained in a single experiment. Similar results were obtained in two (A, C, and D) or four (B) other experiments. All experiments were performed in the presence of tiotidine (30 μM) to eliminate the cAMP response mediated via H_2 receptors.

measured using a sensitive protein binding assay (22) a similar decay was still observed (n = 5; data not shown). This effect was more obvious when a lower dose of adenosine (10 μM) was used. In these experiments, cAMP levels did not even achieve steady state, but started to fall after approximately 5 min (fig. 5). This fall was much faster than that seen when a similar level of cAMP was achieved with 0.1 mM adenosine alone (Fig. 5) or when this higher concentration of adenosine was used in combination with 1 mM histamine (Fig. 4D). The rate of fall therefore does not seem to be related primarily to the level of cAMP.

The reduction in cAMP levels cannot be explained by desensitization of the histamine H_1 receptor. Evidence for this was provided by the studies in Fig. 6, which show that the cAMP response to a combination of histamine (1 mM) and adenosine (0.1 mM) was not altered if the tissue was exposed to histamine for an extra 30 min before adenosine addition. Furthermore, the response to histamine alone in the absence of tiotidine (i.e., an H_1 receptor-mediated augmentation of the response to H_2 receptor stimulation) remained constant over a 45-min period (n = 3; data not shown).

Evidence presented earlier also suggests that desensitization of adenosine receptors is not involved (Fig. 2). This was confirmed by experiments in which a second dose of adenosine (10 μM) was added once the response to adenosine (10 μM) plus histamine (1 mM) had fallen virtually to basal levels. This produced a second cAMP response of similar magnitude to the first (Fig. 7) indicating that the receptors were still active.

The most likely explanation for the decay in the response to histamine plus a low concentration of adenosine is that the adenosine is being removed by uptake and/or metabolism. A removal process with a relatively high affinity for adenosine would cause the adenosine concentration to fall relatively faster when low concentrations of adenosine are used and thus produce a faster decay of the cAMP response in this condition (Fig. 5). Consistent with this hypothesis is the observation that the responses to 10 μM adenosine plus 1 mM histamine and to 10 μM adenosine alone decayed over a similar time course (data not shown), the effect being more obvious in the condition with histamine, due to the amplifying effect of histamine on cAMP levels. Removal of adenosine would also explain why the cAMP response to histamine (1 mM) plus adenosine (10 μM) decayed, while a combination of histamine (1 mM) plus the stable adenosine analogue 2CA (3 μM), which produced a similar initial cAMP response, remained fairly constant (Fig. 8).

Our attempts to confirm that the adenosine was being removed were unsuccessful. Deoxycoformycin (10 μM), an inhibitor of ADA, failed to prevent the fall in cAMP levels (data not shown). This dose of deoxycoformycin was sufficient to prevent added ADA (1.2 units/ml) terminating the effect of 0.1 mM adenosine on cAMP levels. This suggests that the main mechanism removing extracellular adenosine is uptake. Our findings

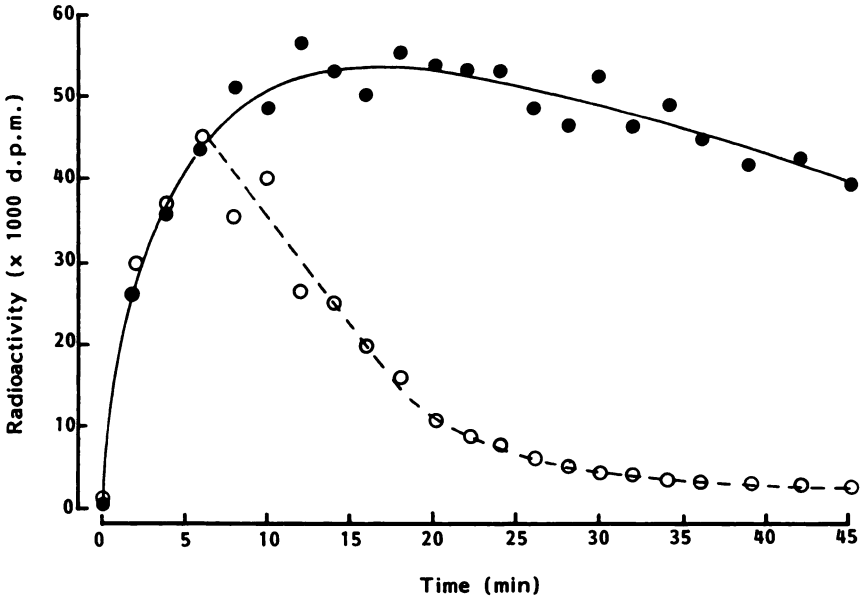

Fig. 5. Time course of the accumulation of [^{3}H]cAMP after addition of 0.1 mM adenosine (●) or 10 μM adenosine plus 1 mM histamine (○) to guinea pig cerebral cortical slices. Incubations were performed in the presence of 30 μM tiotidine. Data shown are from one experiment of three giving similar results.

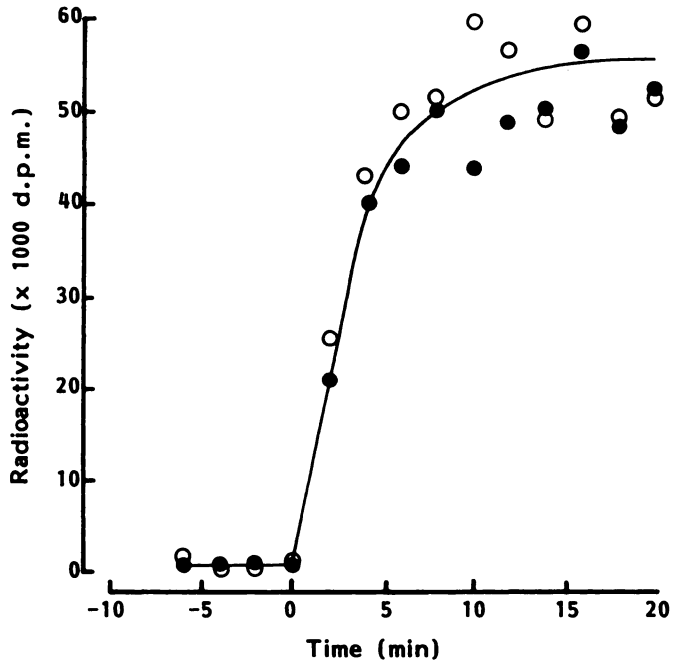

Fig. 6. Time course of the cAMP response to histamine (1 mM) plus adenosine (0.1 mM). ●, Histamine and adenosine added simultaneously at 0 min; ○, adenosine added at 0 min but histamine added 30 min earlier. The experiment was performed in the presence of tiotidine (30 μM). Data were obtained in a single experiment. Similar results were obtained on two other occasions.

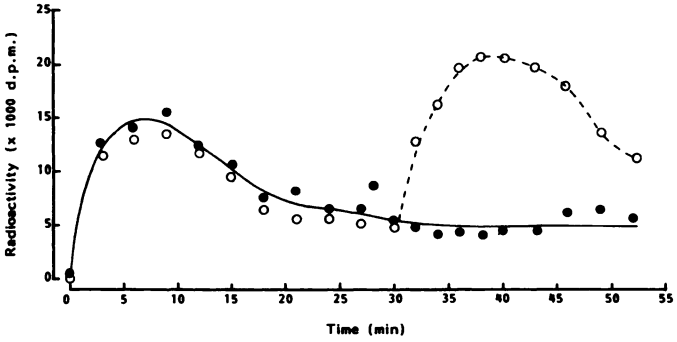

Fig. 7. Time course of the cAMP response to 10 μM adenosine plus 1 mM histamine in guinea pig cerebral cortical slices. Histamine plus adenosine were added at 0 min. ○ indicates that a second dose of 10 μM adenosine was added 30 min later and ●, that it was not. Incubations were performed in the presence of tiotidine (30 μM). Data were obtained in a single experiment. Similar results were obtained in one other experiment.

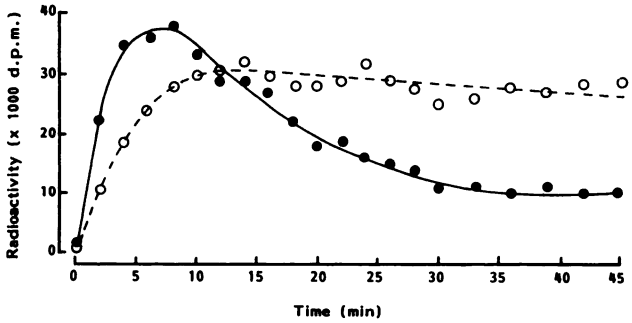

Fig. 8. Time course of the cAMP response to 0.01 mM adenosine plus 1 mM histamine (●) or 3 μM 2CA plus 1 mM histamine (○) in guinea pig cerebral cortical slices. Incubations were performed in the presence of tiotidine (30 μM). Data were obtained in a single experiment. Similar results were obtained in one other experiment.

with the adenosine uptake inhibitors dipyridamole (0.5 μM) are not inconsistent with this. This compound elevated the cAMP response to histamine plus adenosine, as would be expected if the external adenosine concentration had been increased somewhat by a reduction in adenosine uptake. However, this effect could also be a result of the other action of dipyridamole, PDE inhibition, so that this result is inconclusive.

Mode of action of histamine H_1 receptor stimulation. The H_1 receptor-mediated augmentation of the cAMP response to adenosine in cerebral cortical slices may be caused either by a histamine-induced increase in cAMP synthesis and/or a reduction in its breakdown by PDE. To distinguish between these possibilities, an indirect method was used, based on measurement of the rate of decay of tissue cAMP content after rapid removal of the adenosine stimulus.

The rationale of this approach is as follows. In general:

$$\text{Rate of change in tissue cAMP} = \upsilon_{prod} - \upsilon_{break} \quad (1)$$

where υ_{prod} and υ_{break} are the rates of cAMP synthesis and breakdown, respectively. If cAMP synthesis is stopped, υ_{prod} becomes zero and tissue cAMP content will decay at a rate equal to υ_{break}. If, in addition, tissue cAMP content is at steady state when the stimulus is removed, then at this time:

$$(\upsilon_{prod})_s = (\upsilon_{break})_s \quad (2)$$

The initial rate of decay of tissue cAMP content in this case is therefore also equal to $(\upsilon_{prod})_s$. Thus, by comparing initial rates of decay of cAMP content from steady state in slices incubated with and without histamine, effects of histamine on both synthesis and breakdown of cAMP can be detected.

Fig. 1 and Table 1 shows the results of experiments of this sort. Slices were incubated with adenosine (0.1 mM) alone and adenosine (0.1 mM) plus histamine (1 mM) and when steady state had been reached the adenosine stimulus was removed with ADA (1.2 units/ml; see Fig. 1). The absolute initial rate of fall of tissue cAMP was greater in slices incubated with histamine plus adenosine than in slices with adenosine alone (Fig. 1; Table 1). Histamine therefore must have increased the rate of cAMP synthesis. This result contrasts with the findings with the PDE inhibitor rolipram using a similar method approach. Although rolipram increased the steady level of cAMP in brain slices stimulated with adenosine, it had no effect on rate of decay of tissue cAMP content after ADA addition, indicating that it had not altered cAMP synthesis (17).

It follows from Eq. (2) that $(\upsilon_{break})_s$ must also have been increased in the presence of histamine. However, in general, υ_{break} will depend not only on the properties (e.g., V_{max} and K_m) of the relevant PDE enzymes but also on the tissue cAMP concentration. The higher $(\upsilon_{break})_s$ could therefore be due simply to greater stimulation of the relevant PDEs by the higher steady state level of cAMP in the condition with histamine. To determine whether histamine has also altered the properties of the PDEs, it is therefore best to compare the rates of decay in tissue cAMP content at equivalent cAMP levels in slices incubated with and without histamine.

Such a comparison in Fig. 1 suggests that histamine has little effect on the properties of the PDEs inasmuch as the slopes of the cAMP decay curves for cAMP levels of 50,000 dpm and below are rather similar in the two conditions. However, the paucity of experimental points on these parts of the curves makes accurate comparison of slopes difficult. An alternative approach is that used in the experiments of Fig. 9. Here, a low dose of an adenosine receptor agonist was identified that, when combined with 1 mM histamine, produced the same level of cAMP as a higher dose of adenosine agonist alone. The adenosine was then rapidly removed at this point and the decay in tissue cAMP content followed. If the presence of histamine does not alter the properties of the PDEs, the cAMP levels should follow identical time courses in the two conditions.

Fig. 9A shows such an experiment in which matching cAMP accumulations were achieved with 0.1 mM adenosine and 10 μM adenosine plus 1 mM histamine. After addition of ADA to remove adenosine rapidly, the decays in cAMP levels in the two conditions are almost superimposable, indicating little effect of histamine on the properties of the PDEs involved.

It was shown earlier that, with the lower dose of adenosine used in this experiment (10 μM), cAMP levels decay quite rapidly even in the absence of ADA (Fig. 5). This should not invalidate the interpretation of Fig. 9A because all that is required is similar cAMP levels in the two conditions when the adenosine stimulus is removed. However, to counter this possible objection, the experiment was repeated using 2CA instead of adenosine so that a steady level of cAMP would be achieved (see Fig. 8) before stimulus removal. Because 2CA is not metabolized by ADA, it was necessary to use an adenosine antagonist to remove the stimulus. We had initially avoided using such compounds because many are also PDE inhibitors (23–25). However, 8PT has been reported to be a weak inhibitor of PDE (26). This was confirmed in preliminary studies showing that 8PT (0.2 mM) did not elevate the cAMP response to histamine in cerebral cortical slices.

Fig. 9B shows the result of this experiment. The cAMP response to 0.1 mM 2CA was matched with a combination of 1.9 μM 2CA and 1 mM histamine (Fig. 9B). After addition of 8PT (0.2 mM) the decays of cAMP in the two conditions were identical. This therefore provided further evidence against an effect of histamine on the properties of the PDEs.

It is worth noting that the data of Fig. 9 support the conclusion from Fig. 1 and Table 1 that histamine must stimulate cAMP synthesis. Because histamine has no measurable effect on cAMP breakdown, this is the only way by which it can lead to formation of similar steady levels of cAMP with the widely differing concentrations of adenosine or 2CA used in the experiments shown in Fig. 9.

TABLE 1

Comparison of the rates of breakdown of cAMP in guinea pig cerebral cortical slices after removal of the adenosine stimulus during cAMP steady states in the presence of adenosine alone or histamine plus adenosine

Slices were incubated with adenosine (0.1 mM) alone or together with histamine (1 mM). The decay in tissue cAMP content was measured after rapid removal of the adenosine stimulus by addition of ADA (1.2 units/ml). The initial rates of cAMP breakdown were calculated from the slopes of the decay curves over the first 2 min after ADA addition.

Expt.	Rates of breakdown of cAMP	
	Histamine + adenosine	Adenosine
	dpm/min	
1	18,000	10,000
2	21,000	14,000
3	19,000	8,000
Mean ± SE	19,333 ± 883	10,667 ± 1,766*

*$p < 0.02$ (paired t test) with respect to the rate of cAMP breakdown for the condition with histamine present.

Discussion

Activation of histamine H_1 receptors resulted in an augmentation of the cAMP response to adenosine in guinea pig cerebral cortical slices. This is in agreement with previous studies (1, 3–5, 17, 27). Studies of the time course of the cAMP response to either adenosine or a combination of adenosine and histamine indicated that the adenylate cyclase is rapidly switched on by these agonists (Fig. 1). Similarly, studies in which the stimulus was removed with ADA indicated a return of cAMP levels to basal with no measurable delay, showing that the cyclase can be rapidly switched off. The rapid rate at which the cAMP level fell after ADA addition also shows that PDE is very active in this tissue. The rates of cAMP turnover, and hence also synthesis, during steady state are therefore very high.

The cAMP responses to 0.1 mM adenosine or 2CA were well

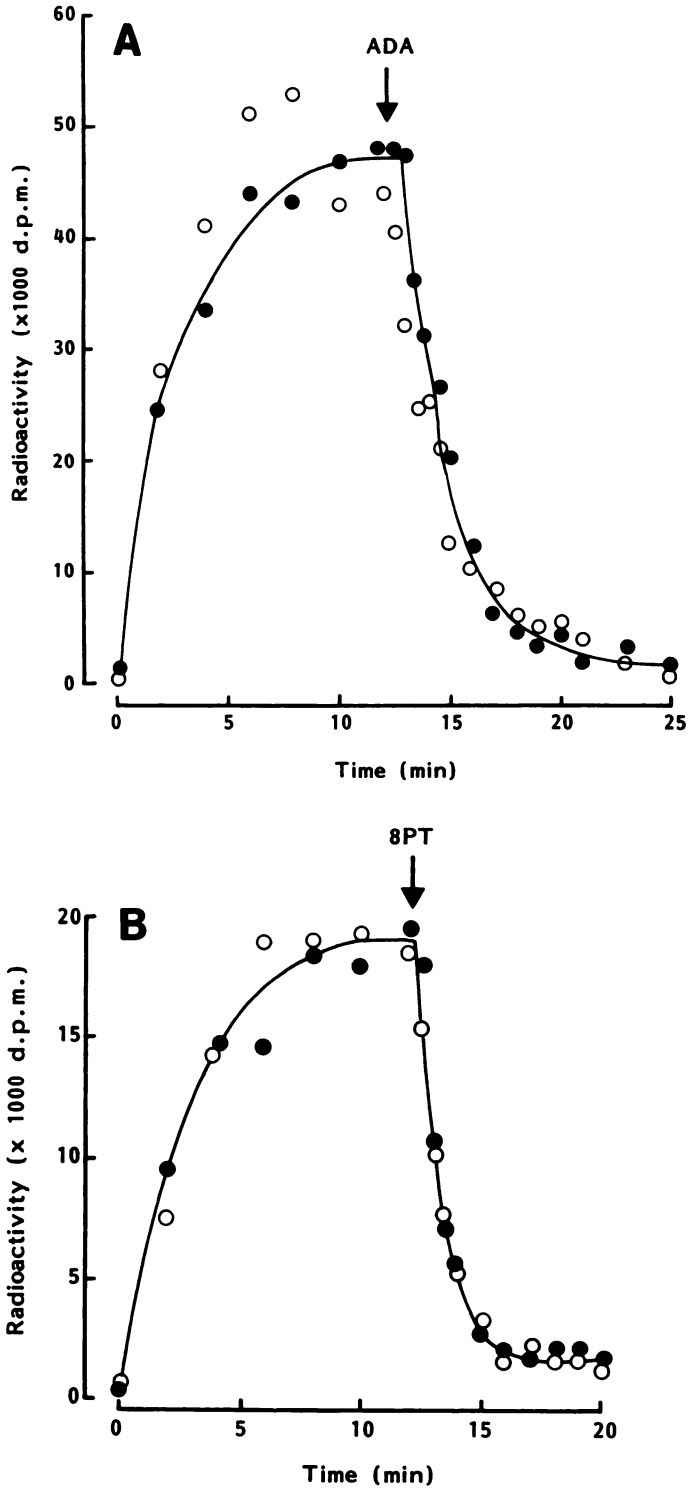

Fig. 9. Comparison of the rates of breakdown of cAMP in guinea pig cerebral cortical slices after rapid removal of the adenosine receptor agonist at cAMP steady state. In A, ●, adenosine (0.1 mM); ○, adenosine (10 μM) plus histamine (1 mM). B, ●, 2CA (0.1 mM); ○, 2CA (1.9 μM) plus histamine (1 mM). The adenosine receptor agonist was removed by addition of 1.2 units/ml ADA (A) or 0.2 mM 8PT (B). Experiments were performed in the presence of 30 μM tiotidine (A and B) and 1.2 units/ml ADA (B). Data were obtained in single experiments. Similar results were obtained on one (A) or two (B) other occasions.

maintained over a 45-min period. This enabled the time course of the H_1 receptor-mediated augmentation to be investigated separately from the direct response mediated by adenosine receptors. After addition of histamine at the adenosine steady state, the onset of the augmentation response was immediate. This suggested that the system mediating the augmentation could be rapidly switched on after receptor activation.

Studies using the H_1 receptor antagonist mepyramine revealed that the continued presence of the histamine stimulus was required to maintain the augmentation, because mepyramine added at any time after histamine addition caused cAMP levels to fall to a level similar to that obtained with adenosine alone (Fig. 4). However, there appeared to be a delay of approximately 2 min after mepyramine addition before cAMP levels started to fall. This gives an upper estimate of the lifetime of any second messenger involved in the augmentation process. Some of the delay may be the time taken for mepyramine to penetrate the tissue and displace histamine from its receptors. However, the delay before cAMP levels started to fall after removal of the adenosine stimulus with 8PT or ADA or removal of histamine H_2 stimulus with tiotidine (17) was always 1 min or less, suggesting that tissue penetration time is probably not a major component. There are insufficient data available about the on- and off-rates for histamine at H_1 receptors to permit reliable estimation of the time required for mepyramine to displace histamine. A more definitive statement on the lifetime of any second messenger involved here therefore cannot yet be made.

The other aspect of histamine's potentiating action on cAMP levels that was studied was the step in the cAMP turnover sequence that is primarily affected. Augmentation of cAMP could be produced by either an increase in cAMP synthesis or a decrease in its breakdown by PDE. Although inhibition of PDE, e.g., by rolipram, can potentiate the adenosine response to a similar degree as H_1 receptor stimulation (17), others have suggested that this is unlikely to be the mechanism by which indirectly acting agents increase cAMP accumulation because potentiation can still be observed in the presence of these inhibitors (28). This argument assumes that PDE inhibitors block PDE activity completely, thus preventing any further reduction in activity as a result of H_1 receptor stimulation. However, many of these inhibitors are competitive and their effect on PDE turnover is therefore likely to be overcome by the increase in cell cAMP concentration that will occur after inhibitor addition when unaltered production of cAMP transiently exceeds its breakdown. Eventually, a new steady state is achieved with a higher cell cAMP concentration, in which cAMP turnover and hence PDE activity is the same as before the inhibitor was added. In this case there is no *a priori* reason to believe that a histamine-induced inhibition of PDE could not still occur and produce a further increase in cAMP level.

Consequently, we used another approach to determine whether an alteration in synthesis or breakdown of cAMP was involved in the augmentation process. cAMP synthesis and breakdown during steady state were assessed by measuring the initial rate of cAMP breakdown after removal of the adenosine stimulus. Two protocols were used that gave consistent and complementary findings, indicating that histamine increases synthesis of cAMP in the presence of adenosine but has little effect on the enzyme(s) responsible for breaking down the cAMP produced in response to these stimuli.

The mechanism by which histamine produces this effect is uncertain although its absence in membrane preparations (9–12) suggests that a second messenger may be involved. One possibility is a change in cell calcium. Removal of external calcium reduces the H_1 receptor-mediated cAMP accumulation in guinea pig cerebral cortex (13) whereas studies in rabbit cerebral cortex have suggested that the H_1 receptor response is mediated by a release of intracellular calcium, which is probably secondary to an increase in the level of inositol trisphosphate (8).

Other agents that might link H_1 receptor occupancy to changes in cAMP synthesis are the products of inositol phospholipid breakdown themselves (the inositol phosphates and diacylglycerol). All of these are produced in response to H_1 receptor activation (21). A role for diacylglycerol has also been suggested by the finding that the cAMP response to 2CA in a synaptoneurosome preparation of guinea pig cerebral cortex is enhanced by phorbol esters, which mimic the effect of diacylglycerol (5). This effect could involve a phosphorylation of adenylate cyclase, because it has been shown recently *in vitro* that protein kinase C (which is activated by diacylglycerol) can phosphorylate the catalytic unit of adenylate cyclase purified from bovine brain (15).

In conclusion, we propose that stimulation of histamine H_1 receptors in guinea pig cerebral cortical slices potentiates the cAMP response to adenosine primarily by increasing cAMP synthesis rather than by decreasing breakdown. This effect occurs rapidly after histamine addition and requires the maintained presence of histamine. It is hoped that the kinetic studies described in the present investigation will provide a basis for further studies to assess the role of putative second messengers in this augmentation response.

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