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Regulation of Adenosine Cyclic 3′,5′-Phosphate Formation in Cerebral Cortical Slices

Interaction among Norepinephrine, Histamine, Serotonin

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SUMMARY

Depolarizing agents (K^+, ouabain, veratridine, and batrachotoxin), biogenic amines (norepinephrine, histamine, and serotonin), and adenosine cause accumulation of ^{14}C-labeled adenosine cyclic 3′,5′-phosphate (cyclic AMP) in cerebral cortical slices that have been labeled in a prior incubation with adenine-^{14}C. The combined effect of supramaximal concentrations of depolarizing agents or adenosine with the biogenic amines is more than additive. The combined effect of supramaximal concentrations of norepinephrine and histamine or of serotonin and histamine is also more than additive. The data obtained are compatible with the existence of compartmentalized pools of adenine nucleotides serving as precursors for cyclic AMP and suggest that these pools are regulated in a synergistic manner by separate "receptors" for adenosine, histamine, and either norepinephrine or serotonin.

INTRODUCTION

A convenient technique for investigating control of the accumulation of adenosine cyclic 3′,5′-phosphate (cyclic AMP) in brain slices consists of labeling pool(s) of adenine nucleotides by prior incubation of slices with radioactive adenine or adenosine and observing the conversion of radioactive nucleotides in the slices to radioactive cyclic AMP during subsequent incubations with depolarizing agents, amines, adenosine, and combinations of these agents (1–5). The results obtained by this method are similar to those obtained by measurements of endogenous levels of cyclic AMP (6, 7), except in the case of stimulation by adenosine (8). The reason for the latter difference appears to be that at the high concentrations of adenosine required for stimulation of cyclic AMP formation, adenosine serves not only as a regulatory stimulant but also as a precursor for cyclic AMP (for discussion, see ref. 5). Investigations using labeled brain slices suggest that a small, compartmentalized pool of adenine nucleotides serves as an excellent precursor for cyclic AMP during incubations with depolarizing agents, adenosine, or biogenic amines (4).

The regulation of cyclic AMP levels in cerebral cortical slices at present has been found to be influenced by three classes of compounds: (a) depolarizing agents, (b) biogenic amines such as norepinephrine,

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histamine, and serotonin, and (c) adenosine and related nucleotides (1–8). At least part of the effect of depolarizing agents on accumulations of cyclic AMP appear to involve the "release" of adenosine (4, 8, 9). The effects on accumulation of cyclic AMP elicited by depolarizing agents or adenosine in combination with biogenic amines are much more than additive (4, 10). Such synergistic interactions suggest the presence of interacting regulatory units for adenyl cyclase, one of which is affected by depolarizing agents via "release" of adenosine, and the other by a biogenic amine. Further studies on accumulation of cyclic AMP in incubated slices of cerebral cortex now provide evidence for regulatory interactions among the biogenic amines (serotonin, histamine, and norepinephrine).

MATERIALS AND METHODS

Male Hartley guinea pigs (350–450 g) were used. All chemicals were obtained from commercial sources. Accumulation of cyclic AMP in brain slices was measured with slices previously labeled with adenine-^{14}C (11.2 μCi/μmole, 13.3 μM) as reported in earlier papers (1–5). Caffeine and EDTA were omitted from the incubation medium.

RESULTS[2]

The time course of the effect of 1 mM histamine, 0.1 mM adenosine, and 43 mM K^+, singly and in combination, on the accumulation of cyclic AMP-^{14}C is shown in Fig. 1. The accumulation of cyclic AMP-^{14}C elicited by this supramaximal concentration of histamine is almost complete after 6–8 min, with only a slight increase occurring during the next 12 min. The effect of a supramaximal concentration of adenosine on cyclic AMP-^{14}C accumulation is also fully expressed after 6–8 min. The effect of 43 mM K^+ on accumulation of cyclic AMP-^{14}C is maximal at 6 min, after which time the

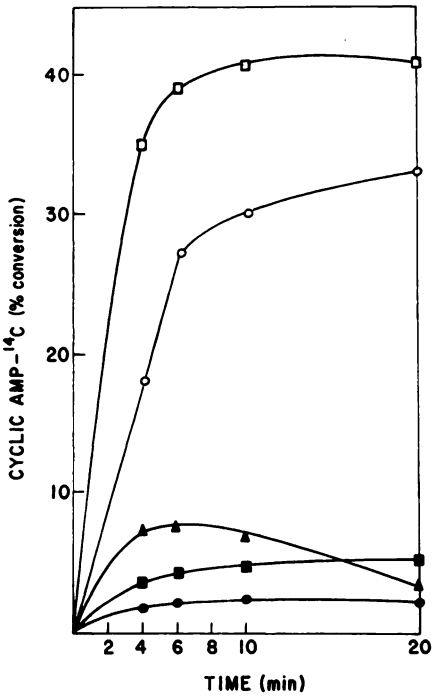

FIG. 1. *Time course of effect of histamine, K^+, and adenosine, singly and in combination, on accumulation of cyclic AMP-^{14}C in labeled slices of guinea pig cerebral cortex*

Cyclic AMP-^{14}C was measured as described previously (1), and the results are expressed as percentage of total radioactive adenine nucleotides in the slices that was converted to cyclic AMP-^{14}C. ■, histamine (1 mM); ▲, K^+ (43 mM); ●, adenosine (0.1 mM); □, histamine and K^+; ○, histamine and adenosine.

[2] It will be noted that depolarizing concentrations of K^+ (40 mM) in this paper afforded 4–5% conversion of nucleotides to cyclic AMP-^{14}C, which is at variance with our previously reported value of 7.8 ± 0.8% (4). This difference was found to be due to variations in the length of time during which brain tissue is maintained in the cold. In the present experiments, the elapsed time from removal of guinea pig brain to initiation of incubation of cortical slices in Krebs-Ringer solution at 37° was 15–20 min, whereas in earlier work (1–4) the elapsed time was less than 10 min. The accumulation of cyclic AMP-^{14}C elicited by veratridine, adenosine, histamine, or norepinephrine or by a combination of K^+ and histamine is not significantly altered by variations in the time of preparation, or even by preparation in Krebs-Ringer solution maintained at 37° rather than at 0–5°. The data in Fig. 1 were obtained with slices prepared in less than 10 min. The same conclusions with regard to synergistic effects are reached if one compares data in terms of counts per minute of cyclic AMP-^{14}C per milligram of nitrogen of the slices.

levels of cyclic AMP-^{14}C decline. The effects of combinations of adenosine and histamine or of K^+ and histamine on accumulation of cyclic AMP-^{14}C are much greater than additive and are again nearly fully expressed within 6–8 min. The effects of these agents on accumulation of cyclic AMP-^{14}C reported in the remainder of this paper have been measured after 7 min, a time at which most of the effects are nearly maximal.

Dose-response curves for the effects of histamine, norepinephrine, and serotonin, both separately and in combination, are shown in Fig. 2. The maximal accumulation of cyclic AMP-^{14}C (0.4 ± 0.2%) elicited by serotonin is not significantly different from the 0.3 ± 0.1% accumulation of cyclic AMP-^{14}C observed in control incubations. Maximal accumulation of cyclic AMP-^{14}C of 0.9 ± 0.2% is elicited by norepinephrine. The responses to combinations of norepinephrine and serotonin were not significantly greater than the response to norepinephrine alone. An ED_{50} value for norepinephrine can be estimated from the dose-response curve in Fig. 2 as approximately 8 μM.

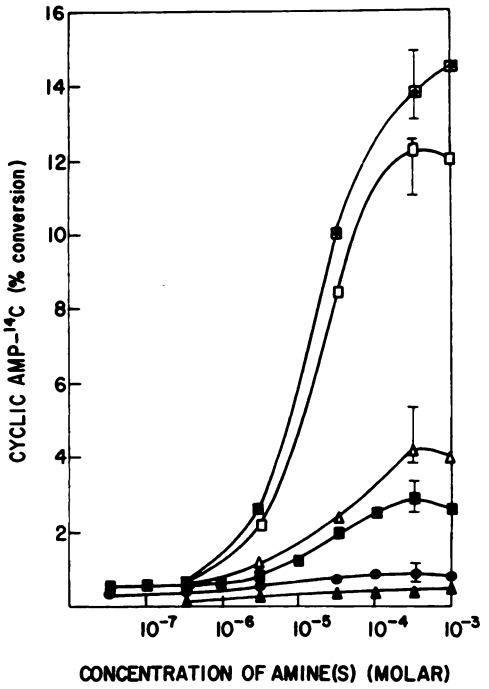

FIG. 2. *Dose-response curves for effects of histamine, norepinephrine, and serotonin, singly and in combination, on accumulation of cyclic AMP-^{14}C in labeled slices of guinea pig cerebral cortex*

Cyclic AMP-^{14}C was measured as described previously (1) after incubation for 7 min. ■, histamine; ●, norepinephrine; ▲, serotonin; □, histamine and norepinephrine; △, histamine and serotonin; ⊡, histamine, norepinephrine, and serotonin. Vertical bars indicate standard deviations (four experiments) at 5 mM concentration of the amines.

Maximal accumulation of cyclic AMP-^{14}C of 3.0 ± 0.4% is elicited by histamine (Fig. 2). The ED_{50} for histamine is approximately 20 μM. The maximal accumulation of cyclic AMP-^{14}C elicited by histamine is significantly increased to 4.7 ± 0.8% in the presence of 5 mM serotonin (Fig. 2). The effect of histamine on accumulation was unchanged or slightly decreased in the presence of the same concentration of normetanephrine, phenethanolamine, octopamine, tyramine, or dopamine. The effects of combinations of histamine and norepinephrine on the accumulation of cyclic AMP-^{14}C are much greater than the sum of the individual effects. Thus, the maximal accumulation of cyclic AMP-^{14}C elicited by combinations of these two amines in four experiments was 11.8 ± 0.8%, while the maximal accumulation due to norepinephrine or histamine separately was 0.9 ± 0.2% and 3.0 ± 0.4%, respectively. The additional presence of serotonin caused a further increase in the accumulation of cyclic AMP-^{14}C to 14.0 ± 0.9%.

The accumulation of cyclic AMP-^{14}C elicited by 0.1 mM histamine and 0.1 mM norepinephrine were increased from 2.0 to 2.8% and from 0.7 to 1.0%, respectively, by the presence of 1 mM cocaine. The accumulation of cyclic AMP-^{14}C elicited by the combination of 0.1 mM histamine and 0.1 mM norepinephrine was also increased from 9.8 to 12.0% by the presence of 1 mM cocaine.

The dose-response curves for the effects of histamine, norepinephrine, and serotonin on the accumulation of cyclic AMP-^{14}C in the presence of depolarizing concentrations of K^+ or in the presence of 0.1 mM adenosine are shown in Fig. 3. These curves were obtained in the presence of 40 mM K^+, with

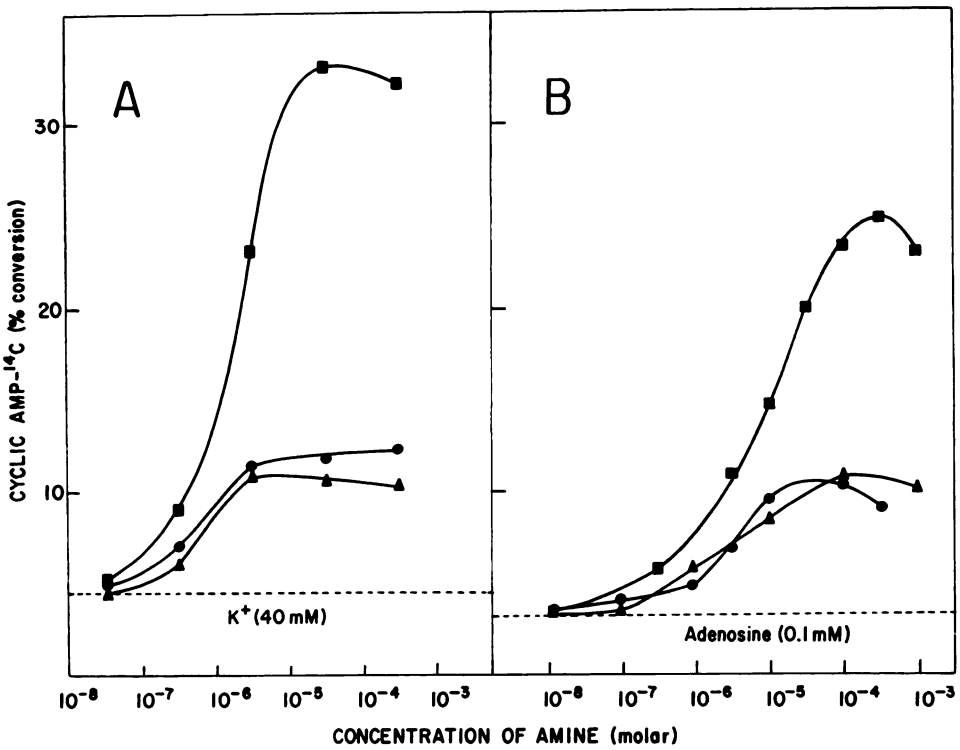

FIG. 3. *Dose-response curves for effects of biogenic amines on accumulation of cyclic AMP-^{14}C in labeled slices of guinea pig cerebral cortex in the presence of K^+ (A) and adenosine (B)*

Cyclic AMP-^{14}C was measured as described previously (1) after incubation for 7 min. ■, histamine; ●, norepinephrine; ▲, serotonin. Incubation with 40 mM K^+ alone (A) resulted in 4.4% conversion of radioactive nucleotides to cyclic AMP-^{14}C; incubation with 0.1 mM adenosine alone (B) yielded 3.0% conversion.

which 4.4 ± 0.4% accumulation of cyclic AMP-^{14}C was observed (Fig. 3A, dashed line), or of 0.1 mM adenosine, with which 3.0 ± 0.3% accumulation of cyclic AMP-^{14}C occurred. Maximal accumulation of cyclic AMP-^{14}C of approximately 11% was observed with either serotonin or norepinephrine in the presence of 40 mM K^+. In the presence of 0.1 mM adenosine, these amines also elicited maximal accumulation of cyclic AMP-^{14}C of approximately 11%. Histamine elicited a 33 ± 3% (four experiments) accumulation of cyclic AMP-^{14}C in the presence of 40 mM K^+, and 25 ± 3% (four experiments) in the presence of 0.1 mM adenosine. The approximate ED_{50} values for histamine, norepinephrine, and serotonin in the presence of 40 mM K^+ are 2 μM, 0.8 μM, and 0.8 μM, respectively, while in the presence of adenosine the ED_{50} values appear larger; i.e., 10 μM, 4μM, and 5 μM, respectively. The ED_{50} values for norepinephrine and histamine in normal Krebs-Ringer medium also appear to be larger; i.e., 20 μM and 8 μM, respectively.

The combined effects of submaximal concentrations (with respect to accumulation of cyclic AMP-^{14}C) of histamine, norepinephrine, and serotonin in the presence of depolarizing concentrations of 40 mM K^+ or in the presence of 0.1 mM adenosine are presented in Table 1. The effects of these combinations of amines were in all cases additive or greater than additive (experiments 1, 2, 4, and 5, Table 1). For example, in experiment 1, histamine caused a 9.8% increase in percentage accumulation of cyclic AMP-^{14}C in the presence of 40 mM K^+. Norepinephrine or serotonin caused a 1.4–1.8% increase, and a combination of hista-

TABLE 1

Effects of biogenic amines on accumulation of cyclic AMP in slices of guinea pig cerebral cortex in the presence of adenosine or K^+

Labeled slices of guinea pig cerebral cortex were incubated for 7 min, and cyclic AMP-^{14}C was measured as described previously (1). Results are reported as percentage conversion of total adenine-^{14}C nucleotides in the slices to cyclic AMP-^{14}C.

Additions	Conversion to cyclic AMP-^{14}C				
	With 40 mM K^+			With 0.1 mM adenosine	
	Expt. 1	Expt. 2	Expt. 3[a]	Expt. 4	Expt. 5
	%	%	%	%	%
None	3.0	3.5	4.4	3.0	3.2
Histamine, 5 μM	12.8	12.6	27.0	11.8	11.5
Norepinephrine, 1 μM	4.4	4.8	12.0	4.8	4.8
Serotonin, 1 μM	4.8	6.4	14.1	5.8	5.8
Histamine + norepinephrine	16.5	15.6	28.4	14.2	
Histamine + serotonin	16.2	18.6	28.4	17.9	
Histamine + norepinephrine + serotonin	18.3	18.7	29.6	18.7	
Norepinephrine + serotonin	7.6	8.4	19.1	9.7	

[a] In experiment 3, the concentrations of the amines were 0.1 mM.

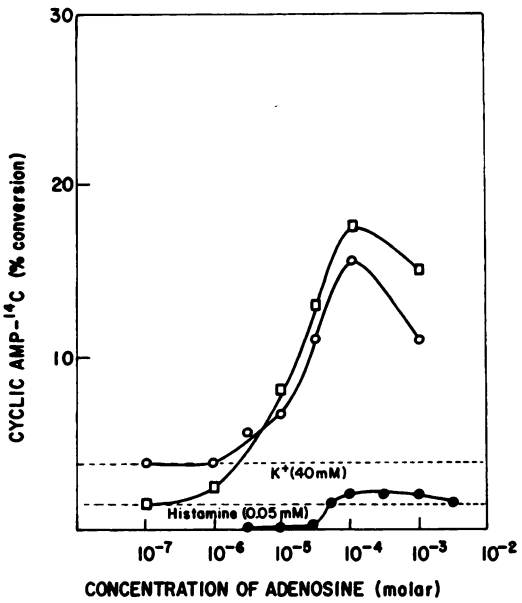

FIG. 4. *Dose-response curves for effects of adenosine, alone and in the presence of either K^+ or histamine, on accumulation of cyclic AMP-^{14}C in labeled slices of guinea pig cerebral cortex*

Cyclic AMP-^{14}C was measured as described previously (1) after incubation for 7 min. ●, adenosine; □, adenosine and 50 μM histamine; ○, adenosine and 40 mM K^+. Incubation with K^+ alone and with histamine alone resulted, respectively, in 3.8% and 1.5% conversion of radioactive nucleotides to cyclic AMP-^{14}C.

mine with either norepinephrine or serotonin caused a 13.2–13.5% increase (greater than additive). Similarly, the combined effect of norepinephrine and serotonin results in a 3.6% increase in accumulation of cyclic AMP-^{14}C (greater than additive). It is perhaps of some importance that the effects of serotonin both alone and in combination with norepinephrine or histamine are much more striking in the presence of either 40 mM K^+ or adenosine (Table 1 and Fig. 3) than in their absence (Fig. 2). Indeed, the effect of serotonin under these conditions is equal to or greater than that of norepinephrine.

Experiment 3 of Table 1 presents the results of higher concentrations (0.1 mM) of these amines, both alone and in combination, in the presence of 40 mM K^+. In this type of experiment, the combined effects were either additive (norepinephrine plus serotonin) or less than additive (all other combinations). This may reflect the fact that 30–35% conversion of adenine-^{14}C nucleotides in the slice represents the maximum attainable conversion of the precursor pool. Histamine alone under the conditions

of experiment 3 caused 27% conversion, which could be increased to only 29.6% by the additional presence of histamine and norepinephrine.

Dose-response curves for the effect of adenosine by itself, in the presence of histamine, or in the presence of depolarizing concentrations of K^+ are shown in Fig. 4. As is readily apparent, the effect of adenosine is greatly potentiated, and to almost the same extent, with either 40 mM K^+ or 0.05 mM histamine. In both cases, an accumulation of approximately 16% occurs, while in normal Krebs-Ringer solution adenosine elicits only a 2.2 ± 0.3% accumulation of cyclic AMP-^{14}C. The ED_{50} for adenosine appears somewhat smaller (20–40 μM) in the presence of histamine or 40 mM K^+ than in normal Krebs-Ringer solution (70 μM).

DISCUSSION

Previous studies on the accumulation of cyclic AMP-^{14}C in labeled slices of cerebral cortex indicated that synergistic effects between biogenic amines and depolarizing agents or adenosine are a common phenomenon (4, 10). It has been postulated that at least part of the stimulatory activity of depolarizing agents in causing accumulation of cyclic AMP is due to the participation of "released" adenosine, which interacts with a regulatory unit for adenyl cyclase (9). The synergism with depolarizing agents and amines occurs with histamine and norepinephrine and even with serotonin, an amine that by itself has negligible activity in stimulating accumulation of cyclic AMP-^{14}C in the cerebral cortex. Evidence for separate regulatory receptors for histamine and norepinephrine in rabbit cerebellum and cerebral cortex has been reported (6, 7). In view of the published data, it was reasonable to propose that compartmentalized pools of adenine nucleotides, which serve as precursors for cyclic AMP, exist in the cerebral cortex and that many of these pools have two interacting regulatory units governing adenyl cyclase activity: one for adenosine and one for a biogenic amine, either histamine, norepinephrine, or serotonin. The present paper, however, provides evidence for interactions among these biogenic amines. For example, the effects on accumulation of cyclic AMP-^{14}C of histamine and norepinephrine, or of histamine and serotonin, are greater than additive at both sub- and supramaximal concentrations (Fig. 2). The synergistic effect observed with norepinephrine is not due to its activity as a depolarizing agent (11), since cocaine, a "membrane stabilizer," which prevents depolarization, did not block the synergism. Cocaine instead slightly potentiated the effects of low concentrations of either norepinephrine or histamine, probably because of its action in blocking amine uptake mechanisms (12). That the synergistic effect between histamine and serotonin was due to a nonspecific effect on the metabolism or uptake of histamine in the presence of serotonin seems unlikely, since a variety of other amines, such as β-phenethanolamine, normetanephrine, octopamine, tyramine, and dopamine, did not potentiate histamine. None of these amines has any significant effect on the accumulation of cyclic AMP-^{14}C in cerebral cortex (2, 4).[3] The effect of the three stimulatory amines (histamine, norepinephrine, and serotonin) together was slightly greater than that due to a combination of histamine and norepinephrine (Fig. 2). The combined effects of submaximal levels of the biogenic amines in the presence of 40 mM K^+ or of 0.1 mM adenosine are additive rather than synergistic (Table 1), suggesting that the synergism between amines is maximally effective only when the adenosine-sensitive regulatory unit is not activated. Maximal conversion of labeled nucleotides to cyclic AMP-^{14}C of 30–35% can be elicited in slices of guinea pig cerebral cortex. This accounts for the lack of additivity seen when supramaximal levels of histamine in the presence of 40 mM K^+ are incubated along with supramaximal levels of either norepinephrine or serotonin (Table 1, experiment 3).

Differences in the apparent ED_{50} values for histamine and norepinephrine with regard to stimulated accumulation of cyclic AMP-^{14}C in control medium versus media containing either adenosine or depolarizing

[3] Unpublished observations.

concentrations of K^+ (Figs. 2 and 3) might reflect allosteric changes in the amine-sensitive regulatory units for adenyl cyclase as a result of interaction of adenosine or adenosine "released" by depolarization with the adenosine-sensitive regulatory unit. The converse might also be true, since it appears that lower concentrations of adenosine are effective in stimulating accumulation of cyclic AMP in the presence of histamine (Fig. 4). Other explanations involving metabolism of cyclic AMP or transport phenomena might, however, pertain. Similar differences in ED_{50} values, such as shown in Figs. 2–4, were seen in at least two sets of experiments. The ED_{50} values for histamine, norepinephrine, and adenosine in guinea pig cerebral cortex, determined by the present method, are similar to values reported previously [respectively, 10 μM (rabbit), 5 μM (rabbit), and 30 μM (guinea pig)], based on changes in endogenous levels of cyclic AMP in incubated slices of cerebral cortex (7, 8). The pronounced synergistic effect between histamine and norepinephrine reported here was not noted in earlier studies on the effect of these amines on endogenous levels of cyclic AMP in rabbit cerebellum and cerebral cortex (6, 7). Those studies were performed in the presence of 0.5 mM theophylline. However, this concentration of theophylline does not prevent the synergism between histamine and either serotonin or norepinephrine with regard to accumulation of cyclic AMP-^{14}C in guinea pig cerebral cortex.[3] Preliminary attempts to demonstrate synergism between histamine and norepinephrine in slices of rabbit cerebral cortex by the prior labeling technique were unsuccessful. Combinations of the two amines did result in greater than additive effects on accumulation of cyclic AMP-^{14}C, but the results were not statistically significant.[3] The failure in these preliminary experiments and in the studies by Kakiuchi and Rall (7) to demonstrate additive or more than additive effects of histamine and norepinephrine in rabbit cerebral cortex may have been due to the great difference in individual effects of these amines in this species. The effects of combinations of histamine and norepinephrine or of histamine and serotonin on accumulation of cyclic AMP-^{14}C in rat cerebral cortex were more than additive.[3]

The present observations on synergistic interactions between biogenic amines such as histamine and norepinephrine, or histamine and serotonin, and previously published reports of synergism between adenosine and biogenic amines (4, 10) indicate that pools of adenine nucleotides exist in cerebral cortex, whose conversion to cyclic AMP is catalyzed by an adenyl cyclase responsive to separate but interacting regulatory units for adenosine, histamine, and norepinephrine, or adenosine, histamine, and serotonin. This is in contrast to results reported for adipose cells using the same labeling technique. In that preparation it appeared that each agent (norepinephrine, glucagon, corticotropin, etc.) could fully activate adenyl cyclase (13). The subtle nature of various interactions of agents affecting the accumulation of cyclic AMP in the central nervous system remains open to many interpretations. It is hoped that further investigations will clarify the reasons for these subtleties, as well as providing evidence for the role of cyclic AMP in the central nervous system.

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