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Inhibition of Glutamate-Elicited Accumulation of Adenosine Cyclic 3',5'-Monophosphate in Brain Slices by α,ω-Diaminocarboxylic Acids

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SUMMARY

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The accumulation of cyclic 3',5'-AMP elicited by glutamate in incubated slices of guinea pig cerebral cortex was inhibited by 2,3-diaminopropionate, 2,4-diaminobutyrate, or ornithine. The inhibitory action of histidine was marginal, and other basic amino acids, ω-monoamino acids, and aliphatic diamines were ineffective as inhibitors. 2,3-Diaminopropionate at 2 mM inhibited the stimulatory effect of glutamate more than 50% with respect to both endogenous and radioactive cyclic AMP; the latter was derived from intracellular nucleotides labeled during a prior incubation with radioactive adenine. Such a marked inhibitory action of 2,3-diaminopropionate was not observed toward the effects of adenosine, histamine, or a histamine-norepinephrine combination. 2'-Deoxyadenosine was found to be capable of inhibiting the adenosine effect, using either the radioactive labeling assay or the measurement of endogenous levels of cyclic AMP. The glutamate effect was not inhibited by the nucleoside. The results demonstrate that 2,3-diaminopropionic acid and 2'-deoxyadenosine are specific antagonists of the respective receptors, the former for acidic amino acids and the latter for adenosine, both of which mediate accumulation of cyclic AMP in incubated brain slices.

INTRODUCTION

The content of adenosine cyclic 3',5'-monophosphate in incubated brain slices is elevated by three classes of endogenous compounds: (a) adenosine and related nucleotides (1, 2), (b) certain acidic amino acids like glutamate and aspartate (3-5), and (c) biogenic amines, such as catecholamines and histamine (6, 7). The effects of both adenosine and the acidic amino acids are markedly inhibited by various methylxanthines, but those of biogenic amines are not. The maximal effects of certain acidic amino acids like cysteinesulfinic acid are greater with respect to cyclic AMP accumulation than that of adenosine, while the two classes of compounds elicit an additive effect when added in combination at their optimally effective concentrations (3). These and other findings obtained in our laboratory (5) indicate that the site of action of the acidic amino acids is likely to be different not only from that of biogenic amines but also from that of adenosine. Before discovery of the effect of the acidic amino acids, methylxanthines had been thought to be specific antagonists of the adenosine receptor (1, 8). Therefore a final conclusion concerning the occurrence of an acidic amino acid receptor associated with cyclic AMP elevation had to be reserved until discovery of specific antagonists of the respective receptors, one for adenosine and the other for acidic amino acids. The evidence presented in this paper shows that certain ω-amino-α-amino acids

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may be considered specific antagonists of the acidic amino acid receptor, whereas deoxyadenosines, but not methylxanthines, are specific antagonists of the adenosine receptor.

MATERIALS AND METHODS

Chemicals. 1,3-Diaminopropane, DL-2,3-diaminopropionic acid, and L-2,4-diaminobutyric acid were purchased from Tokyo Kasei Chemicals; L-dihydroxyphenylalanine and kainic acid, from Wako Chemicals; DL-*o*-tyrosine, putrescine, DL-β-aminoisobutyric acid, α-methyl-*m*-tyrosine, DL-phenylserine, 2′-deoxyadenosine, 3′-deoxyadenosine, and α-methyl-DL-glutamic acid, from Sigma; 1-methyl-3-isobutylxanthine, from Aldrich; and *p*-chloro-L-phenylalanine, from Fluka AG. Ibotenic acid was a gift from Dr. C. H. Eugster, Zurich University, and L-ornithine, L-histidine, and L-lysine were obtained from Ajinomoto (Japan). L-Glutamate γ-ethyl ester, L-methioninesulfoximine, α-methyl-DL-ornithine, *N*-*p*-hydroxybenzyl-β-alanine, and L-glutamate α,γ-diethyl ester were synthesized in our laboratory according to known procedures. The identities of the synthesized compounds were confirmed from their nuclear magnetic resonance spectra, and purity was ascertained by various systems of chromatography. Sources of other compounds were described in the preceding papers (3, 5).

Incubation of slices. Male Hartley guinea pigs (280 ± 20 g) were stunned and bled from the carotid artery, and their brains were removed and placed in cold Krebs-Ringer-bicarbonate buffer, pH 7.3. The buffer contained the following constituents: NaCl, 122 mM; KCl, 3 mM, $MgSO_4$, 1.2 mM; $CaCl_2$, 1.3 mM; KH_2PO_4, 0.4 mM; D-glucose, 10 mM; and $NaHCO_3$, 25 mM. Slices 250 μm thick were prepared as rapidly as possible from cortical gray matter with a McIlwain tissue chopper, followed by immediate incubation in the Krebs-Ringer-bicarbonate buffer, which was gassed throughout the experiments with 95% O_2–5% CO_2 at 37°. All test agents were dissolved in the buffer to result in pH 7.3 ± 0.15 by adding either NaOH or HCl, if necessary. Inhibitors were added 2 min prior to addition of stimulatory agents throughout this experiment.

Measurement of cyclic AMP accumulation. Accumulation of cyclic AMP in brain slices was measured by two different methods. The first method was to label an adenine nucleotide pool in slices by incubation with [^{14}C]adenine and subsequently to determine rates of conversion from total [^{14}C]nucleotides in slices to cyclic [^{14}C]AMP during incubation with various agents (7). The second method was to estimate levels of endogenous cyclic AMP in slices, per milligram of protein, according to Gilman's protein binding method (9), after incubation with various agents. The details of the two methods have been described previously (3). In most instances remarkably similar results have been obtained by the two methods (3, 10). Since both methods measure the net accumulation of cyclic AMP in slices, not only enhanced synthesis but also inhibited degradation of cyclic AMP could lead to accumulation. Adenosine might act as an indirect inhibitor of cyclic 3′,5′-phosphodiesterase to some extent, but its major mechanism of action appears to be activation of the cyclic AMP-generating system (10). Glutamic and cysteinesulfinic acids lack inhibitory action on phosphodiesterase activity in the range of concentrations used (3).

RESULTS

Antagonists of acidic amino acid receptor. Based on the receptor hypothesis proposed in a previous report (Figs. 2 and 3 of ref. 5), we have searched for possible antagonists of the acidic amino acid receptor among those compounds which can be accommodated conformationally to the receptor model, retaining at least two of the three active ionized groups of the agonists. γ-Aminobutyric acid, β-alanine, DL-β-aminoisobutyric acid, *N*-*p*-hydroxybenzyl-β-alanine, 2-amino-3-phosphonopropionic acid, α-methyl-DL-glutamic acid, taurine, glycine, succinic acid, pyruvic acid, L-glutamate γ-ethyl ester, L-glutamate α,γ-diethyl ester, L-methioninesulfoximine, L-phenylalanine, *p*-chloro-L-phenylalanine, DL-phenylserine, L-dihydroxyphenylalanine, L-tyrosine, DL-*o*-tyrosine, and α-methyl-*m*-tyrosine were tested at 2 mM using the labeling method described in MA-

TERIALS AND METHODS. All these compounds turned out to lack significant inhibitory action toward the cyclic [^{14}C]AMP accumulation elicited by a 2 mM concentration of L-glutamate. Then a series of ω-amino-α-amino acids were tested, under identical conditions, as possible inhibitors of the glutamate-elicited accumulation of cyclic [^{14}C]AMP. 2,4-Diaminobutyrate was found to be the most powerful inhibitor, followed by DL-2,3-diaminopropionate, L-ornithine, and DL-α-methylornithine (Table 1). The inhibitory action of L-histidine was marginal, and that of L-lysine was not demonstrable. These inhibitory compounds (2 mM) alone did not stimulate cyclic AMP formation. Other basic amino acids, i.e., L-citrulline and L-arginine, and aliphatic diamines such as 1,3-diaminopropane and putrescine were also without inhibitory action under these conditions (data not shown).

The time course of the inhibitory action of 2,3-diaminopropionate toward the glutamate-elicited formation of cyclic [^{14}C]AMP is illustrated in Fig. 1. When examined after 8 min of incubation, 2,3-diaminopropionate inhibited the glutamate response in a hyperbolic fashion (Fig. 2). In other words, the stimulatory effect of 0.8 mM glutamate was virtually unaffected by varying concentrations of diaminopropionate, whereas the latter compound inhibited the effect of 5 mM glutamate in a concentration-dependent manner.

The stimulatory effect of other amino acids, e.g., aspartate and cysteinesulfinate, at 2 mM was also inhibited markedly

TABLE 1

Inhibition of glutamate-elicited formation of cyclic [^{14}C]AMP by various α,ω-diaminocarboxylic acids

Slices were incubated with 3.7 μM [^{14}C]adenine, washed, and divided among several beakers containing inhibitors dissolved in 10 ml of Krebs-Ringer buffer. After incubation of the labeled slices for 2 min in the presence and absence of the inhibitor, L-glutamate dissolved in 0.2 ml of Krebs-Ringer buffer and adjusted to pH 7.3 with NaOH was added to each beaker to result in a final concentration of 2 mM, and incubation was continued for 8 min. Cyclic [^{14}C]AMP formed during the incubation was estimated as described (3), and expressed as percentage conversion, which refers to the percentage of cyclic [^{14}C]AMP compared to the total radioactivity present in the slices. Results are averages of four experiments, ± standard error. Control values (without glutamate) were 0.3 ± 0.1%.

Inhibitor	Cyclic [^{14}C]AMP	Inhibition
	% conversion	%
None	4.4 ± 0.6	
DL-2,3-Diaminopropionate, 2 mM	2.3 ± 0.4	51
L-2,4-Diaminobutyrate, 1 mM	3.0 ± 0.6	34
L-2,4-Diaminobutyrate, 2 mM	1.9 ± 0.4	61
L-Ornithine, 2 mM	2.8 ± 0.2	38
α-Methyl-DL-ornithine, 2 mM	3.1 ± 0.5	32
L-Lysine, 2 mM	4.6 ± 0.7	0

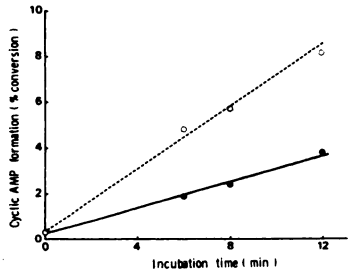

FIG. 1. *Time course for glutamate-elicited formation of radioactive cyclic AMP.*

Labeled slices were incubated in the absence (○---○) and presence (●——●) of 2 mM 2,3-diaminopropionate. The concentration of glutamate was 2.5 mM.

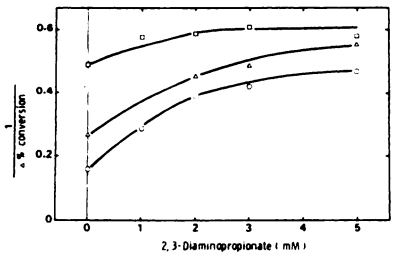

FIG. 2. *Dixon plot of 2,3-diaminopropionate inhibition of glutamate-elicited accumulation of radioactive cyclic AMP.*

Formation of cyclic [^{14}C]AMP was expressed as percentage conversion as described in MATERIALS AND METHODS. An average control value of 0.27% was subtracted from each value before plotting. Incubation was carried out for 8 min with L-glutamate (□, 0.8 mM; △, 2 mM; ○, 5 mM).

by the same concentration of either 2,3-diaminopropionate or 2,4-diaminobutyrate. In contrast, diaminopropionate did not inhibit the cyclic [^{14}C]AMP elevation elicited with other classes of stimulants, i.e., adenosine, histamine, and a histamine-norepinephrine combination, at their optimal and suboptimal concentrations (1, 6, 7, 10) (Table 2). Diaminobutyrate showed a weak inhibitory action toward the adenosine-elicited elevation of cyclic [^{14}C]AMP when tested at 2 mM, but not at 0.2 mM (Table 2).

The labeling method (the radioassay of cyclic [^{14}C]AMP) employed in these screening and kinetic studies has been shown to give results consonant with those obtained by measurement of levels of endogenous cyclic AMP in brain slices incubated with various agents, except for a few cases of quantitative inconsistency (3, 10). In order to confirm that 2,3-diaminopropionate is a specific antagonist of the acidic amino acid receptor, the stimulatory effects of glutamate, adenosine, and histamine on accumulation of endogenous cyclic AMP were tested in the presence and absence of diaminopropionate (Table 3). The result was essentially the same as that obtained with the labeling method. Diaminopropionate at 2 mM inhibited the stimulatory response elicited with equimolar glutamate by about 50%, but did not have a comparable effect on the responses elicited with optimally effective concentrations of adenosine and histamine.

Agonists of acidic amino acid receptor. Every potent agonistic amino acid investigated so far, such as aspartic, glutamic, and cysteinesulfinic acids, possesses an acidic group at both ends of an aliphatic carbon chain and one amino group at the α-position. The antagonism study described above indicated that replacement of the ω-acidic group of the agonists with an amino group creates an antagonist. Therefore these structure-activity relationship studies concerning both the agonists

TABLE 2

Effects of 2,3-diaminopropionate and 2,4-diaminobutyrate on formation of cyclic [^{14}C]AMP elicited by various stimulants

Formation of cyclic [^{14}C]AMP in the presence and absence of the inhibitor, 2 mM, was measured as described in Table 1. Results of separate experiments were normalized to the average effect of 2 mM L-glutamate, which was included in each experiment as a reference. The normalized values were then expressed as means ± standard errors. Numbers in parentheses refer to the number of experiments performed.

Stimulant	Inhibitor		
	None	2,3-Diaminopropionate	2,4-Diaminobutyrate
		% conversion	
None	0.2 ± 0.1 (5)	0.3 ± 0.2 (5)	0.2 ± 0.1 (3)
L-Glutamate, 2 mM	4.3 ± 0.3 (13)	2.3 ± 0.4 (4)	1.9 ± 0.4 (4)
L-Glutamate, 5 mM	5.8 ± 0.6 (4)	2.4 ± 0.5 (3)	
L-Aspartate, 2 mM	4.3 ± 0.7 (3)	2.5 ± 0.5 (3)	1.8 ± 0.5 (3)
L-Cysteinesulfinate, 2 mM	10.9 ± 1.3 (5)	5.4 ± 0.9 (4)	5.1 ± 1.2 (4)
		4.6 ± 0.5[a] (3)	3.9 ± 0.8[a] (3)
Adenosine, 0.03 mM	2.4 ± 0.4 (5)	2.5 ± 0.6 (5)	
Adenosine, 0.5 mM	5.9 ± 0.5 (6)	5.8 ± 0.4 (4)	4.3 ± 0.3[b] (5)
			6.1 ± 0.4[c] (4)
Histamine, 0.1 mM	2.1 ± 0.3 (4)	2.0, 1.9 (2)	2.1 ± 0.4 (3)
Histamine, 1 mM	3.2 ± 0.5 (8)	3.3 ± 0.4 (4)	3.5 ± 0.6 (3)
Histamine, 1 mM, + norepinephrine, 0.5 mM	8.0 ± 1.0 (3)	7.9 ± 0.8 (3)	7.8 ± 0.8 (3)

[a] The concentrations of the inhibitors were 5 mM, instead of 2 mM.

[b] Lower than control; $p < 0.05$.

[c] Concentration of the inhibitor was 0.2 mM.

TABLE 3

Effect of 2,3-diaminopropionate on accumulation of endogenous cyclic AMP in cerebral cortical slices during incubation with various stimulatory agents

Slices were incubated for 25 min and divided among several beakers containing 2,3-diaminopropionate, 2 mM, unless otherwise specified, or the buffer alone. After 2 min of incubation the stimulatory agent was added and incubation was continued for 8 min. The content of cyclic AMP was assayed as described in MATERIALS AND METHODS. Values are means ± standard errors of the number of experiments in parentheses.

Stimulant	No inhibitor	2,3-Diaminopropionate	Inhibition
	pmoles cyclic AMP/mg protein		*%*
None	15 ± 2 (5)	17 ± 4 (4)	
L-Glutamate, 2 mM	185 ± 16 (4)	104 ± 11 (4)	49
L-Glutamate, 5 mM	433 ± 29 (3)	142 ± 8 (3)	70
L-Aspartate, 2 mM	172 ± 11 (4)	115 ± 20 (3)	39
Adenosine, 0.03 mM	198 ± 9 (3)	190 ± 13 (3)	4[a]
Adenosine, 0.5 mM	507 ± 29 (5)	478 ± 30 (5)	6[a]
Histamine, 0.1 mM	90, 88 (2)	112, 115 (2)	0
Histamine, 1 mM	121 ± 15 (4)	136 ± 19 (4)	0
Histamine, 1 mM, + norepinephrine, 0.5 mM	505 ± 28 (3)	498 ± 31 (3)	2[a]

[a] Not statistically significant.

and antagonists are in favor of the receptor model for the acidic amino acids with three ionized centers (see ref. 5). In order to test this hypothetical model further, we examined the effects of kainic acid and ibotenic acid as possible agonists of the receptor. These heterocyclic compounds are structurally much more restricted than the aliphatic acidic amino acids but have three ionized groups that might be accommodated to the receptor model. The two compounds were in fact effective, although less so than L-glutamate, in elevating cyclic [^{14}C]AMP in cerebral cortical slices (Table 4). However, the stimulatory effect of the two conformationally restricted compounds was not inhibited by 2,3-diaminopropionate (Table 4). The latter finding, together with the observation of a diaminopropionate-resistant component in the glutamate effect, suggests that the effect of acidic amino acids may actually consist of two components, of which both kainic and ibotenic acids possess the resistant component alone.

Antagonists of adenosine receptor. The inhibitory action of both 2′- and 3′-deoxyadenosine toward the adenosine-elicited formation of radioactive cyclic AMP in labeled brain slices was previously reported by Huang *et al.* (11). The inhibitory action of 0.5 mM 2′-deoxyadenosine on the response to adenosine was confirmed using both the labeling assay and the measurement of endogenous cyclic AMP in incubated brain slices. Unlike the inhibition of the glutamate effect by 2,3-diaminopropionate, inhibition of the adenosine effect by 2′-deoxyadenosine gave a straight line in a Dixon plot over a range of inhibitor concentrations between 0.1 and 0.5 mM (Fig. 3).

TABLE 4

Stimulatory effects of kainic and ibotenic acids, alone and in combination with 2,3-diaminopropionate, on formation of cyclic [^{14}C]AMP

Formation of cyclic [^{14}C]AMP was measured as described in Table 1. Results are averages ± standard errors of three experiments

Addition	Cyclic [^{14}C]AMP formation
	% conversion
None	0.3 ± 0.1
Kainic acid, 2 mM	2.3 ± 0.9
Kainic acid, 5 mM	3.2 ± 0.6
Kainic acid, 5 mM, + 2,3-diaminopropionate, 2 mM	3.0 ± 0.6
Kainic acid, 10 mM	3.6 ± 0.8
Ibotenic acid, 2 mM	2.6 ± 0.7
Ibotenic acid, 2 mM, + 2,3-diaminopropionate, 2 mM	2.5 ± 0.4
Ibotenic acid, 10 mM	4.0 ± 1.1
L-Glutamate, 2 mM	4.1 ± 0.8
L-Glutamate, 10 mM	6.9 ± 1.2

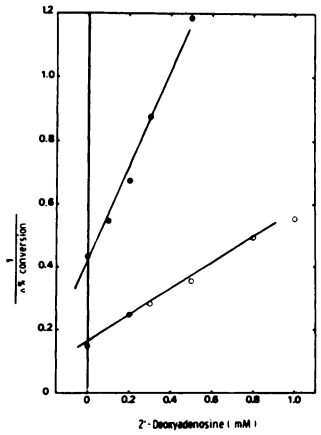

FIG. 3. *Dixon plot of 2'-deoxyadenosine inhibition of adenosine-elicited accumulation of radioactive cyclic AMP.*

Adenosine concentrations were 0.03 mM (●) and 0.1 mM (○). See Fig. 2 for other details.

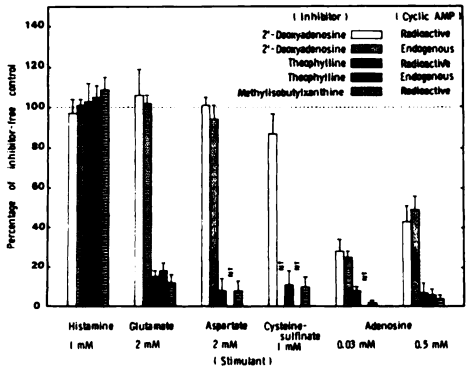

FIG. 4. *Effect of 2'-deoxyadenosine and methylxanthines on accumulation of radioactive and/or endogenous cyclic AMP elicited by various stimulatory agents.*

2'-Deoxyadenosine (0.5 mM) or methylxanthines (1 mM) were added 2 min before the stimulatory agent. Incubation was terminated 8 min after addition of the stimulatory agent, and radioactive and endogenous cyclic AMP was determined as described in Tables 1 and 3, respectively. Results are reported as the mean percentage ± standard error of the response elicited by the stimulatory agent alone. NT, not tested.

The effect of ATP was antagonized by deoxyadenosine to almost the same extent as the effect of adenosine, while the effect of neither amino acids nor biogenic amines was influenced by deoxyadenosine when examined with the labeling method or the endogenous cyclic AMP assay (Fig. 4 and Table 5). Even a high concentration (5 mM) of deoxyadenosine did not inhibit the response to 2 mM glutamate (Table 5). Both the diaminopropionate-resistant component of the glutamate effect and the effect of kainic acid were also not antagonized by deoxyadenosine (Table 5). On the other hand, theophylline and 1-methyl-3-isobutylxanthine both inhibited the responses to adenosine and glutamate without inhibiting the histamine response (Fig. 4).

Adenosine and biogenic amines produced a synergistic effect, as reported previously by various investigators. 2'-Deoxyadenosine abolished the potentiation effect of adenosine nearly completely (Table 5).

DISCUSSION

2,3-Diaminopropionic acid and 2'-deoxyadenosine were found to be specific antagonists of acidic amino acid and adenosine receptors, respectively, both of which mediate accumulation of cyclic AMP in incubated cerebral cortical slices of guinea pig. Thus diaminopropionate at 2 mM inhibited the cyclic AMP accumulation elicited with equimolar L-glutamate by nearly 50% while this inhibitor concentration was virtually ineffective toward the stimulated accumulation of the nucleotide elicited with lower, yet optimal, concentrations of histamine or adenosine. A series of α,ω-diaminocarboxylic acids, such as 2,4-diaminobutyrate and ornithine, appear to belong to this group of acidic amino acid receptor antagonists. 2,4-Diaminobutyrate at high concentrations also was revealed to possess a weak inhibitory action toward adenosine (Table 2). 2,3-Diaminopropionate and ornithine[1] probably lack inhibitory action toward adenosine and therefore may be considered to be more specific for the acidic amino acid receptor. However, it was impossible to obtain complete inhibition of the glutamate effect by diaminopropionate, as was shown by the hyperbolic inhibition pattern of the Dixon plot (Fig. 2). In addition, the effects of kainic and ibotenic acids were not significantly antagonized by diaminopropionate (Table 4). It

[1] Our preliminary result did not show a significant inhibitory action of L-ornithine (2 mM) toward the stimulatory effect of adenosine (0.2 mM).

TABLE 5

Inhibitory effect of 2'-deoxyadenosine on formation of cyclic AMP stimulated by various agents

Formation of cyclic AMP was measured by the labeling method (see Table 1 for details). The concentration of 2'-deoxyadenosine, which was added 2 min prior to stimulants, was 1.0 mM unless otherwise specified. Results are averages ± standard errors of three or four experiments.

Other additions	Formation of cyclic [^{14}C]AMP	
	Without deoxyadenosine	With deoxyadenosine
	% conversion	
None	0.28 ± 0.05	0.25 ± 0.05
Histamine, 0.5 mM	3.6 ± 0.4	3.5 ± 0.6
Norepinephrine, 0.5 mM	1.9 ± 0.2	2.0 ± 0.3
Adenosine, 0.1 mM, + histamine, 0.5 mM	20.1 ± 2.7	4.0 ± 1.1
Adenosine, 0.1 mM, + norepinephrine, 0.5 mM	17.2 ± 2.7	3.3 ± 1.0
Adenosine, 0.1 mM	5.8 ± 0.4	1.6 ± 0.3
ATP, 0.1 mM	5.6 ± 0.9	1.8 ± 0.3
Kainic acid, 2 mM	2.3 ± 0.9	2.3 ± 0.6
Kainic acid, 5 mM	3.2 ± 0.6	3.0 ± 0.8
Glutamate, 0.8 mM	1.9 ± 0.3	2.0 ± 0.3
Glutamate, 2 mM, + 2,3-diaminopropionate, 5 mM	2.1 ± 0.2	2.8 ± 0.4
Glutamate, 2 mM	4.2	4.1[a]

[a] The concentration of deoxyadenosine for this experiment was 5 mM.

is likely, therefore, that the effect of acidic amino acids on cyclic AMP accumulation may actually consist of two components: one inhibitable with 2,3-diaminopropionate, and the other a resistant component. Both kainic and ibotenic acids may possess only the resistant component of the effect, while the marked effects of cysteinesulfinate and glutamate at high concentrations appear to be manifested by the diaminopropionate-inhibitable component. The question of the relationship between the receptor model and the two components remains to be solved.

2'-Deoxyadenosine meets the criteria for an antagonist of the adenosine receptor, in that the nucleoside strongly inhibits the adenosine-elicited accumulation of cyclic AMP without inhibiting the response to either glutamate or histamine. In our preliminary study 3'-deoxyadenosine behaved in like manner with respect to the specific inhibition to adenosine. Together with 2,3-diaminopropionate, the two deoxyadenosines can be used to distinguish the acidic amino acid receptor from the adenosine receptor. The present findings, however, by no means indicate that the acidic amino acid receptor is completely independent from the adenosine receptor, because both theophylline and 1-methyl-3-isobutylxanthine blocked the two receptor responses equally effectively (Fig. 4).

Pull and McIlwain (12) reported recently that L-glutamate at 5 mM increased the release of adenine nucleotides from incubated, superfused cerebral cortical tissues and suggested that the increase of cyclic AMP content by glutamate might be brought about by intermediation of released adenosine. The possibility is made very unlikely, however, by the present finding that 2'-deoxyadenosine inhibited the adenosine-elicited accumulation of cyclic AMP without inhibiting the response to either glutamate or histamine. Also contradicting this possibility are the observations that glutamate and adenosine stimulate cyclic AMP accumulation in an additive manner (3) and that the effects of certain acidic amino acids are greater than the maximal effect of adenosine (5). The question arose, then, whether the 2,3-diaminopropionate-resistant component, if not all, of the glutamate effect may be mediated by adenosine. This possibility was also negated by the present finding that neither the effect of kainic acid

nor the resistant component of the glutamate effect was antagonized by deoxyadenosine (Table 5).

In the present study with the two conformationally restricted analogues of glutamic acid, kainic acid and ibotenic acid, the stimulatory activity of both compounds was less potent than that of L-glutamate. This might be explained, on the basis of the recptor model, by the facts that the acidity of the ω-acidic group of ibotenic acid is weaker than that of the ω-carboxyl group of glutamic acid and that the isopropenyl moiety of kainic acid tends to hinder sterically its ω-carboxyl group. On the contrary, however, both kainic and ibotenic acids are much stronger than glutamic acid as excitants of central neurons when applied iontophoretically (13–15). It is interesting in this context that none of the antagonists of glutamate- or aspartate-elicited excitation of central neurons, such as γ-aminobutyrate, glycine, glutamate γ-ethyl ester, and methioninesulfoximine (16, 17), showed any antagonism of the acidic amino acid receptor mediating cyclic AMP accumulation. Since the glutamate effect on cyclic AMP levels is not blocked by cocaine or tetrodotoxin (3) in spite of reports that the depolarizing effect of iontophoretically applied glutamate is completely blocked by tetrodotoxin (16, 18), it now appears very unlikely that the glutamate-elicited accumulation of cyclic AMP is a consequence of the depolarizing action of glutamate. Considering all these findings together, glutamate and aspartate can be classified as a new class of endogenous compounds which elicit accumulation of cyclic AMP in brain slices. Although the concentrations of the amino acids used in this experiment were relatively high, the optimal concentration for the amino acids might be lowered if a broken cell preparation were used instead of slices (19).

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