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# Adenosine-Sensitive Adenylate Cyclase in Rat Striatal Homogenates and Its Relationship to Dopamine- and $Ca^{2+}$-Sensitive Adenylate Cyclases

JOËL PRÉMONT, MICHÈLE PEREZ, AND JOËL BOCKAERT

*Laboratoires de Physiologie Cellulaire, 75231 Paris Cedex 05, France*

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SUMMARY

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Rat striatal homogenates were found to contain adenosine-sensitive adenylate cyclase, which could be fully stimulated by endogenous adenosine under standard adenylate cyclase assay conditions. Adenosine present in the adenylate cyclase assay had two origins, the homogenate and ATP hydrolysis during incubation. Theophylline, caffeine, and isobutylmethylxanthine inhibited the adenosine-sensitive adenylate cyclase. At low concentrations, theophylline inhibition was competitive ($K_I$ = 20 $\mu$M). The adenosine present in the assay could be removed by using adenosine deaminase. It was then possible to stimulate the adenosine-sensitive adenylate cyclase by adenosine analogues resistant to adenosine deaminase treatment, such as 2-chloroadenosine, $N^6$-phenylisopropyladenosine, and 5′-deoxyadenosine, which was a partial agonist. Adenine, inosine, 5′-AMP, and ATP were neither agonists nor antagonists. In addition to adenosine-sensitive adenylate cyclase, rat striatal homogenates contain $Ca^{2+}$- and dopamine-sensitive adenylate cyclases. Each of these three adenylate cyclase systems was found to be independent of the others.

## INTRODUCTION

Adenosine seems to play an important regulatory role in mammalian adenylate cyclase activity. Adenosine enhances the cyclic 3′,5′-AMP content of brain slices (1, 2), nerve cell lines (3–5), fibroblasts (6), and bone cells (7), and reduces it in fat cells (8). Brain slices do not permit easy analyses of the mechanism by which adenosine controls cyclic AMP production. Indeed, in intact brain tissue, adenosine is specifically taken up (9, 10) and metabolized (9), and may even modulate the release of other substances, such as neurotransmitters (11). These are probably some of the reasons why the remarkable synergistic effect on cyclic AMP production of the addition of adenosine and either biogenic amines (9), glutamate, aspartate, or depolarizing drugs (9) is still poorly understood. Prevention of the control of both the metabolism of adenosine and its possible indirect effect on cyclic AMP production is thus necessary to understand the mechanism of adenosine action.

This report demonstrates the presence and specificity of adenosine-sensitive adenylate cyclase in a cell-free preparation of rat striatum. The relationships between

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this adenylate cyclase and the dopamine- and $Ca^{2+}$-sensitive adenylate cyclases in rat striatal homogenates were also examined.

## MATERIALS AND METHODS

*Homogenate preparation.* Male Sprague-Dawley rats weighing 350–500 g were killed by decapitation at 2 p.m. Their brains were removed, and their striata were dissected on a refrigerated plate (4°) with glass manipulators. Tissues were homogenized (five strokes), using a Dounce homogenizer, in 2 mM Tris-maleate, pH 7.2, and 2 mM EGTA[1] (usually two striata in 3 ml) at 4°. Homogenates were then filtered through a silk screen (150-$\mu$m pore diameter).

*Adenylate cyclase assay.* Adenylate cyclase activity was measured by the conversion of [$\alpha$-$^{32}$P]ATP to cyclic [$^{32}$P]AMP. The reaction mixture (final volume, 100 $\mu$l) contained 25 mM Tris-maleate (pH 7.2), 0.25 mM ATP, 1 mM $MgSO_4$, 0.4 mM EGTA (added with the homogenate), 0.2 mg/ml of creatine kinase, 20 mM creatine phosphate, and, where indicated, 0.4 IU/ml of adenosine deaminase.

The reaction was initiated by adding the homogenate (20 $\mu$l). After 2 min at 30°, 1 $\mu$Ci of [$\alpha$-$^{32}$P]ATP and 0.001 $\mu$Ci of cyclic [$^3$H]AMP were added and the reaction was allowed to proceed for 5 min at 30°. The reaction was then stopped by addition of 100 $\mu$l of a solution containing 5 mM ATP, 5 mM cyclic AMP, 50 mM Tris-HCl (pH 7.4), and 1% sodium dodecyl sulfate. The cyclic [$^{32}$P]AMP formed and the cyclic [$^3$H]AMP added as a recovery marker were isolated according to Salomon *et al.* (12). The range of variation between independent determinations was less than 10%. All values reported were at least the means of two independent determinations. Adenylate cyclase activities were expressed in picomoles (per minute per milligram of protein). Proteins were determined according to Lowry *et al.* (13), using bovine serum albumin as a standard.

[1] The abbreviations used are: EGTA, ethylene glycol bis($\beta$-aminoethyl ether)-*N*,*N*'-tetraacetic acid; PIA, $N^6$-phenylisopropyladenosine.

*Determination of adenosine concentration in rat striatal homogenates.* Two striata from one rat (80 mg, fresh weight) were homogenized in 1.5 ml of 2 mM Tris-maleate, pH 7.2, and 2 mM EGTA. Then 300 $\mu$l of trichloracetic acid (50%, w/v) were added at 4°. After 20 min the homogenate was centrifuged at 5000 × *g* for 10 min. The trichloracetic acid was extracted three times with 4.5 ml of ethyl ether. Adenosine in the aqueous phase was separated from the phosphate nucleotides as follows. The sample (1 ml) was filtered through a small DEAE-Sephadex A-50 column (1 × 0.5 cm); the eluate contained adenosine and less than 1% phosphorylated nucleoside derivatives. The adenosine concentration was determined from the decrease in optical density at 260 nm induced by adding 0.4 IU/ml of adenosine deaminase in 100 mM phosphate buffer, pH 6.8 (14).

*Chemicals.* ATP (disodium salt), 5'-AMP, 3'-deoxyadenosine, adenosine, 2-chloroadenosine, inosine, adenine, caffeine, papaverine, and theophylline were obtained from Sigma; dopamine and 2'-deoxyadenosine, from Calbiochem; creatine kinase, creatine phosphate, cyclic AMP, and adenosine deaminase, from Boehringer/Mannheim; isobutylmethylxanthine, from Aldrich; and 5'-deoxyadenosine, from P-L Biochemicals. The following drugs were kindly donated: fluphenazine, by Squibb; $N^6$-phenylisopropyladenosine, by Boehringer/Mannheim; (−)-alprenolol, by Geigy; and coformycin, by Dr. H. Umezawa.

## RESULTS

*Inhibition of adenylate cyclase of rat striatal homogenates by methylxanthines or adenosine deaminase.* Papaverine (0.1 mM) inhibits 75% of the phosphodiesterase activity of rat striatal homogenates (15). The addition of 1 mM cyclic AMP to the assay did not significantly increase adenylate cyclase activity (Table 1). In any case, the tracer amount of cyclic [$^3$H]AMP added during incubation permitted correction for the small amount of cyclic AMP hydrolyzed during the adenylate cyclase assay. Under these conditions, methylxan-

TABLE 1

*Effects of cyclic AMP, methylxanthines, and adenosine deaminase on adenylate cyclase of rat striatal homogenates*

The protein concentration was 0.31 and 0.25 mg/ml in experiments 1 and 2, respectively. Results are the means ± standard errors of three determinations.

| Expt | Addition | Concentration | Adenylate cyclase activity |
|---|---|---|---|
| | | *mM* | *pmoles/min/mg* |
| 1 | None | | 310.4 ± 22.6 |
| | Cyclic AMP | 1 | 339.2 ± 21.0 |
| | Theophylline | 0.6 | 130 ± 10.8 |
| | | 5.0 | 106 ± 1.6 |
| | Caffeine | 1.0 | 163.4 ± 3.6 |
| | | 5.0 | 126.8 ± 6.2 |
| | Isobutylmethylxanthine | 0.1 | 197.8 ± 9.8 |
| | | 1.0 | 118.2 ± 3.6 |
| | Adenosine deaminase (0.4 IU/ml) | | 175.4 ± 2.8 |
| | Adenosine deaminase (0.4 IU/ml) + theophylline | 0.6 | 122 ± 9 |
| 2 | None | | 321 ± 17.4 |
| | Adenosine deaminase 0.02 IU/ml | | 193.2 ± 6.8 |
| | 0.1 IU/ml | | 166.6 ± 2.4 |
| | 0.4 IU/ml | | 158 ± 7.8 |
| | 1.0 IU/ml | | 153.2 ± 4.4 |

thines (theophylline, caffeine, and isobutylmethylxanthine) inhibited the adenylate cyclase activity by 60–70% (Table 1). This obviously was not due to the known ability of these compounds to inhibit phosphodiesterases, since such inhibition would increase, not reduce, cyclic AMP accumulation. On the contrary, the inhibitory effect of methylxanthines on the adenylate cyclase activity in our cell-free preparation might, like their inhibitory effect on cyclic AMP accumulation in brain slices (1), be due to blockade of an adenosine-sensitive adenylate cyclase stimulated by endogeneous adenosine. Moreover, adenosine deaminase (0.4 IU/ml), like the methylxanthines, was able to reduce adenylate cyclase activity in striatal homogenates (Table 1), and the effects of methylxanthines and adenosine deaminase were not additive (Table 1). The adenosine deaminase concentration used (0.4 IU/ml) produced a maximal effect (Table 1).

*Competitive effect of low theophylline concentrations on sensitive adenylate cyclase.* In the absence of theophylline, adenosine (1 μM–1 mM) elicited a very small increase (less than 10%) in labeled cyclic AMP production (Fig. 1). The slight inhibition of enzyme activity in the presence of adenosine concentrations higher than 10 μM was probably due to an interaction of adenosine with the catalytic site of the adenylate cyclase. Figure 1 indicates that the inhibition of adenylate cyclase activity by theophylline was concentration-dependent. Adenosine (1 mM) stimulated the adenylate cyclase activity 2-fold in the presence of theophylline (1 mM). As expected for competitive inhibition, the $IC_{50}$ (concentration of theophylline producing half-maximal inhibition) shifted to the right for increasing concentrations of adenosine (Fig. 1). However, the biphasic profile of the theophylline dose-inhibition curve indicated that this drug had a composite effect (Fig. 1). Only the data in Fig. 1 obtained with low theophylline concen-

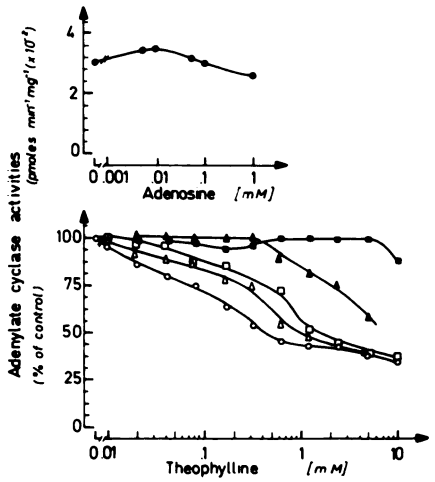


FIG. 1. *Effect of adenosine on adenylate cyclase activity in the absence (upper) and presence (lower) of theophylline*

The effect of theophylline on adenylate cyclase activity was measured at various adenosine concentrations: ■, 1 mM; ▲, 0.1 mM; □, 0.01 mM; △, 0.005 mM; ○, without exogenous adenosine. The protein concentration was 0.28 mg/ml.

trations (less than 0.1 mM) could be plotted to reveal competitive inhibition (curve not shown). Competitive inhibition of the adenosine-sensitive adenylate cyclase by low theophylline concentrations is also clear in the experiment reported in Fig. 4. In this experiment, the apparent affinity of theophylline for the adenosine-sensitive adenylate cyclase was 20 μM. At high concentrations, theophylline produced further inhibition, which did not seem related to blockade of the adenosine site. Indeed, theophylline induced greater inhibition of adenylate cyclase activity than did maximal amounts of adenosine deaminase (Table 1).

*Origin of adenosine in the adenylate cyclase assay.* The adenosine present in the adenylate cyclase assay could have two different origins: the homogenate itself and the ATP added to the adenylate cyclase assay. The adenosine concentration in the homogenates, determined as described under MATERIALS AND METHODS, was 10 ± 2.5 μM ($n$ = 4), corresponding to a final adenosine concentration of 2 μM in the assay. This concentration was high enough to produce submaximal stimulation of the adenosine-sensitive adenylate cyclase. An attempt to eliminate endogenous adenosine by successive centrifugations was apparently unsuccessful (Table 2). After washing the 5000 × $g$ pellet twice, inhibition of the adenylate cyclase activity induced by adenosine deaminase could still be detected. The adenylate cyclase activity measured in the combined presence of adenosine deaminase and 2-chloroadenosine (an agonist of adenosine-sensitive adenylate cyclase and resistant to adenosine deaminase action; see Figs. 2 and 4) was always equivalent to that found in the controls (Table 2). The endogenous adenosine was diluted at least 1000-fold during the entire washing procedure, and the final adenosine concentration was thus expected to be about 1 nM in the assay performed with the $C_3$ 5000 × $g$ pellet. Since under these conditions the adenosine-sensitive adenylate cyclase remains fully stimulated, it is concluded that the source of adenosine, in addition to the homogenate, is also the ATP present in the assay.

TABLE 2

*Effect of washing particulate fractions of rat striatal homogenates on adenosine-sensitive adenylate cyclase activity*

Three milliliters of homogenate prepared as described under MATERIALS AND METHODS were centrifuged for 10 min at 5000 × $g$ at 4°. The pellet ($C_1$) was washed twice with 2 ml of Tris-maleate (2 mM, pH 7.2) and EGTA (2 mM). The particulate fractions obtained after the first and second washes are referred to as $C_2$ and $C_3$, respectively.

| Fraction | Adenylate cyclase activity | | | |
|---|---|---|---|---|
| | Control (I) | Adenosine deaminase (0.4 IU/ml) (II) | Adenosine deaminase +2-chloroadenosine (10 μM) (III) | III:I |
| | *pmoles/min/mg* | | | |
| Homogenate | 309.4 | 143.0 | 280.0 | 0.90 |
| $C_1$ | 233.2 | 131.0 | 224.6 | 0.96 |
| $C_2$ | 207.0 | 136.0 | 206.0 | 0.99 |
| $C_3$ | 224.0 | 150.0 | 200.0 | 0.89 |

The experiment reported in Table 3 was designed to test this assumption. Adenosine could be either a contaminant of ATP or a product of its hydrolysis. The adenosine present in the homogenate and that which may be present in the assay were both deaminated using adenosine deaminase (0.4 IU/ml) in an initial 2-min incubation (Table 3). 2-Chloroadenosine added under these conditions for a second preliminary incubation was still able to stimulate the adenosine-sensitive adenylate cyclase.

Coformycin, a highly potent adenosine deaminase inhibitor, effectively inhibited adenosine deaminase action when added either with this enzyme or later on. When coformycin was present during the second preliminary incubation period, after adenosine deamination as above, the adenosine-sensitive adenylate cyclase was still fully activated (Table 3), whether ATP was present during the first preliminary incubation or only the final incubation period. Furthermore, coformycin (1 mM) did not enhance adenylate cyclase activity in the presence of 0.5 mM theophylline (Table 3). These results indicate that ATP hydrol-

TABLE 3

*Evidence for hydrolysis of unlabeled ATP to adenosine*

The experiment was divided into three periods: two preliminary incubations of 2 and 3 min, respectively, and a final incubation of 5 min. Carrier-free [$\alpha$-$^{32}$P]ATP was added during the final incubation. The concentrations of adenosine deaminase, 2-chloroadenosine, theophylline, and ATP were 0.4 IU/ml, 50 $\mu$M, 0.5 mM, and 0.25 mM, respectively.

| Additions | | | Adenylate cyclase activity | |
|---|---|---|---|---|
| First period | Second period | Final incubation | | |
| | | | *pmoles/min/mg* | % |
| ATP | None | None | 310 | 100 |
| ATP + adenosine deaminase | None | None | 175.2 | 56 |
| ATP + adenosine deaminase | 2-Chloroadenosine | None | 296 | 95 |
| ATP + adenosine deaminase | Coformycin (1 $\mu$M) | None | 288.4 | 93 |
| ATP | Coformycin (1 $\mu$M) | None | 313.6 | 101 |
| ATP + adenosine deaminase + coformycin (1 $\mu$M) | None | None | 306.8 | 99 |
| ATP + theophylline | None | None | 164.2 | 53 |
| ATP + theophylline | Coformycin (1 mM) | None | 186 | 60 |
| None | None | ATP | 352 | 100 |
| Adenosine deaminase | None | ATP | 231.4 | 65 |
| Adenosine deaminase | 2-Chloroadenosine | ATP | 330.0 | 94 |
| Adenosine deaminase | Coformycin (1 $\mu$M) | ATP | 348.4 | 99 |
| None | Coformycin (1 $\mu$M) | ATP | 348.8 | 99 |
| Adenosine deaminase + coformycin (1 $\mu$M) | None | ATP | 358.6 | 102 |

ysis led to adenosine production during incubation with striatal homogenates.

*Specificity of adenosine "receptor" associated with adenylate cyclase.* The specificity of the adenosine "receptor" implicated in the adenylate-sensitive adenylate cyclase was analyzed by using adenylate cyclase activation as a measure of the interaction of the receptor with adenosine analogues or metabolites. A series of deamination-resistant compounds was tested in the presence of adenosine deaminase. 2-Chloroadenosine and $N^6$-phenylisopropyladenosine were found to be full agonists of the system; i.e., in their presence adenylate cyclase activity was equal to that obtained with 10 $\mu$M adenosine in the absence of adenosine deaminase (Fig. 2). The apparent affinities of these two compounds (i.e., concentrations giving half-maximal stimulation) were 1.2 and 3.2 $\mu$M, respectively. 5'-Deoxyadenosine behaved as a partial agonist (Fig. 2). 5'-AMP, adenine, and inosine were neither agonists nor antagonists (Fig. 2). 2'-Deoxyadenosine, which can be deaminated by adenosine deaminase, was tested in the absence of this enzyme. It was found to be a noncompetitive antagonist of adenylate cyclase, since its inhibition was independent of the adenosine concentration (Fig. 3). A similar result was obtained with 3'-deoxyadenosine (data not shown). Theophylline, on the other hand, was found to be a competitive inhibitor of adenosine (Fig. 1) and of 2-chloroadenosine (Fig. 4). A Hofstee plot (16) of the data presented in Fig. 4 yielded an inhibition constant of 20 $\mu$M. Fluphenazine (a dopamine antagonist), phentolamine (an *alpha* adrenergic antagonist), and alprenolol (a *beta* adrenergic blocking agent) were each tested at 10 $\mu$M and had no effect on adenosine-sensitive adenylate cyclase (data not shown).

*$Ca^{2+}$-sensitive adenylate cyclase in rat striatal homogenates.* In the absence of EGTA, adenylate cyclase was slightly stimulated by very low $Ca^{2+}$ concentrations (approximately 10 $\mu$M) and inhibited by $Ca^{2+}$ concentrations above 20 $\mu$M (Fig. 5). EGTA inhibited by 50% adenylate cyclase activity measured in the presence of adenosine deaminase or theophylline ($IC_{50}$ = 60 $\mu$M; data not shown). The inhibitory

effect of EGTA was reversed by the addition of 200 $\mu$M $Ca^{2+}$, and under these conditions adenylate cyclase activity was slightly higher than that measured in the absence of EGTA (Fig. 5). These results suggest the presence of $Ca^{2+}$-sensitive adenylate cyclase in rat striatal homogenates. Furthermore, enough $Ca^{2+}$ is present in these homogenates to induce almost complete activation.

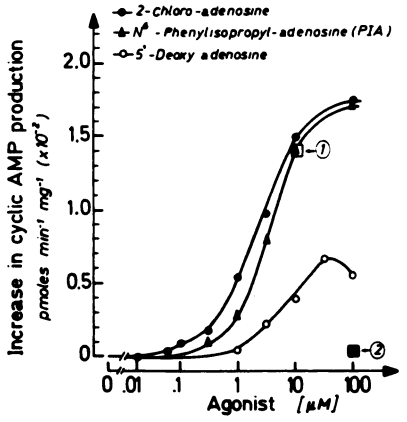


FIG. 2. *Specificity of adenosine-sensitive adenylate cyclase*

The experiment was conducted in the presence of adenosine deaminase (0.4 IU/ml). The adenylate cyclase activity measured in the presence of the adenosine deaminase alone was 175.4 ± 2.8 pmoles/min/mg ($n$ = 3). *1*, adenylate cyclase activity was measured in the presence of 10 $\mu$M $N^6$-phenylisopropyladenosine and either 5'-AMP (100 $\mu$M), adenine (100 $\mu$M), or inosine (100 $\mu$M). *2*, adenylate cyclase activity was measured in the presence of either 5'-AMP, adenine, or inosine, each at 100 $\mu$M. The protein concentration was 0.34 mg/ml. In the absence of adenosine deaminase and the presence of 10 $\mu$M added adenosine, the adenylate cyclase activity was 314.8 ± 4 pmoles/min/mg.

*Absence of any effect of adenosine and $N^6$-phenylisopropyladenosine on dopamine-sensitive adenylate cyclase.* In the presence of adenosine deaminase, the cyclic AMP production induced by maxi-

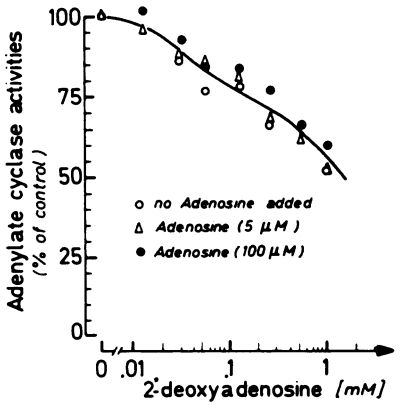


FIG. 3. *Effect of 2-deoxyadenosine on adenosine-sensitive adenylate cyclase*

The adenylate cyclase activities were 300 and 332 pmoles/min/mg in the absence of added adenosine and in the presence of 5 $\mu$M or 100 $\mu$M added adenosine, respectively. The protein concentration was 0.28 mg/ml.

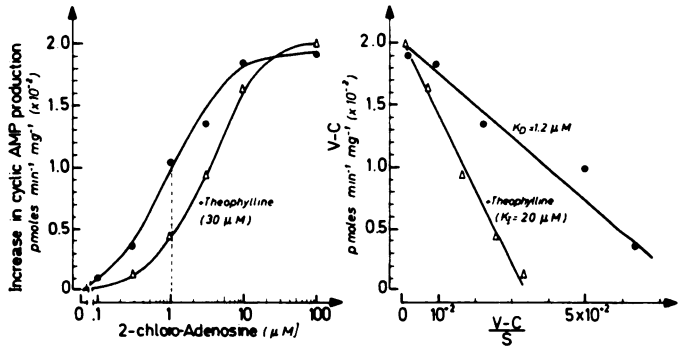


FIG. 4. *Affinity of theophylline for adenosine-sensitive adenylate cyclase*

The dose-response experiments for 2-chloroadenosine were performed in the absence (●) and presence (△) of theophylline. The adenylate cyclase activity measured in the presence of adenosine deaminase was 280 ± 10 ($n$ = 3) pmoles/min/mg. The right-hand part of the figure shows a Hofstee plot (16) of the data. In such plots, the slope of the line is equal to $-K_D$ in the absence of an antagonist and to $-K_D$ (1 + $[I]/K_I$) in the presence of a competitive antagonist, where $[I]$ is the concentration of antagonist and $K_I$ is the affinity of the antagonist for the system. The protein concentration was 0.33 mg/ml.

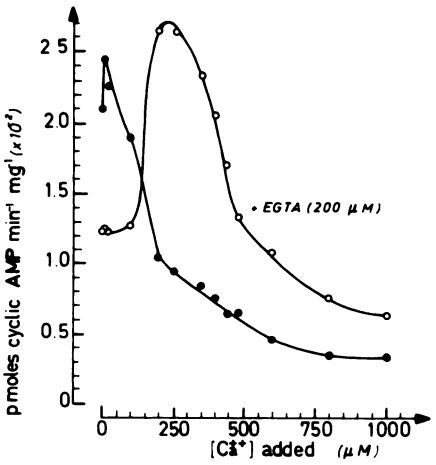


FIG. 5. *$Ca^{2+}$-sensitive adenylate cyclase in rat striatal homogenates*

The homogenates were prepared in the absence of EGTA. The experiment was conducted in the presence of adenosine deaminase (0.4 IU/ml), cyclic AMP (1 mM), theophylline (10 mM), and ATP (1 mM). When present, EGTA was at a final concentration of 200 μM. The protein concentration was 0.360 mg/ml.

mal concentrations of dopamine and PIA was additive. Moreover, the absence or presence of adenosine or PIA did not change the apparent affinity of dopamine for the dopamine-sensitive adenylate cyclase. These observations suggest that these two adenylate cyclases are independent in rat striatal homogenates (Fig. 6).

*Additive effect of adenosine-, dopamine-, and $Ca^{2+}$-sensitive adenylate cyclases from rat striatal homogenates.* In the experiment reported in Fig. 7, basal activity was defined as activity measured in the absence of adenosine (addition of adenosine deaminase, 0.4 IU/ml), dopamine, and free $Ca^{2+}$ (addition of 200 μM EGTA). The $Ca^{2+}$-sensitive adenylate cyclase (Fig. 7, *1*) was estimated according to the cyclic AMP produced under basal conditions when no EGTA was added. The activities of adenosine-sensitive adenylate cyclase (Fig. 7, *2*) and dopamine-sensitive adenylate cyclase (Fig. 7, *4*) were estimated by the increase in cyclic AMP produced in the presence of either PIA or dopamine, respectively. As shown in Fig. 7, the activities of these three adenylate cyclases were additive.

## DISCUSSION

To our knowledge, this is the first report of a direct stimulatory effect exerted by adenosine on adenylate cyclase in a cell-free brain preparation. We were able to demonstrate adenosine stimulation of cyclic AMP production in striatal homogenates only after eliminating the endogenous adenosine present in the assay with adenosine deaminase (Table 1) or displacing it from the adenosine receptor with theophylline (Table 1 and Fig. 1). The adenosine present in the assay originated from both the homogenate and ATP hydrolysis. The adenosine concentration in the homogenate was 10 ± 2.2 μM. The final concentration of the adenosine added with the homogenates was therefore 2 μM. The adenosine in the homogenate either may exist in the striatum *in vivo* or may be derived from ATP hydrolysis during homogenization. Furthermore, in the course of the adenylate cyclase assay, enough adenosine is formed by ATP hydrolysis to stimulate the adenosine-sensitive adenylate cyclase maximally (Tables 2 and 3). This was shown both by the spontaneous reactivation of the adenosine-sensitive

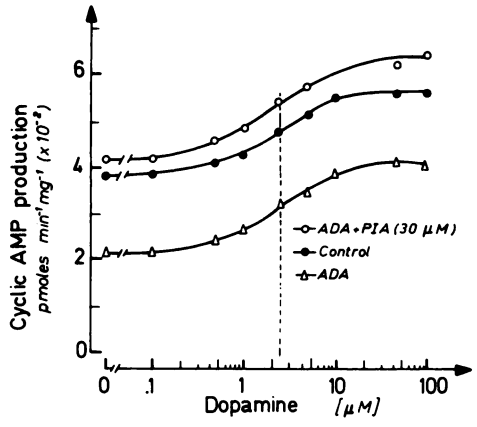


FIG. 6. *Absence of effect of adenosine and PIA on apparent affinity of dopamine for dopamine-sensitive adenylate cyclase*

Three dose-response experiments were performed: in the presence of endogenous adenosine (control; about 2 μM adenosine), adenosine deaminase (ADA) (0.4 IU/ml), and deaminase plus PIA (30 μM). The vertical line indicates the dopamine concentration leading to 50% maximal response. The protein concentration was 0.33 mg/ml.

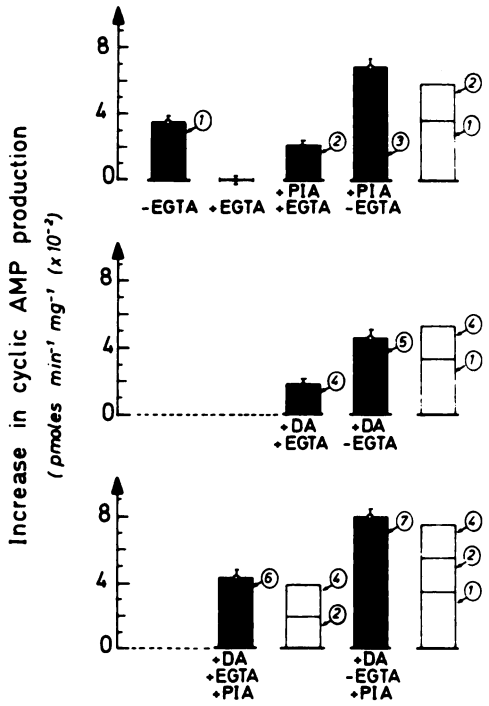


FIG. 7. *Additive effect of $Ca^{2+}$, PIA, and dopamine (DA) on adenylate cyclase activity of rat striatal homogenates*

The increase in cyclic AMP production is equal to the difference between the adenylate cyclase activities measured under the conditions indicated and the adenylate cyclase activity measured in the presence of adenosine deaminase (0.4 IU/ml) and EGTA (0.4 mM) (190 ± 6 pmoles/min/mg). The solid bars indicate the experimental values, and the unfilled bars, the theoretical values expected for each additive effect. Increases in cyclic AMP production due to the presence of: *1*, $Ca^{2+}$; *2*, PIA (30 μM); *3*, $Ca^{2+}$ + PIA; *4*, dopamine (100 μM); *5*, $Ca^{2+}$ + dopamine; *6*, PIA + dopamine; *7*, $Ca^{2+}$ + PIA + dopamine. The protein concentration was 0.36 mg/ml. Values are the means ± standard errors of three determinations.

adenylate cyclase following the action of adenosine deaminase, which was subsequently blocked with coformycin (Table 3), and by the finding that washing the particulate fractions apparently failed to eliminate the adenosine (Table 2).

In any case, adenosine-sensitive adenylate cyclase can be studied in the presence of adenosine deaminase by using adenosine analogues essentially resistant to deamination, such as 2-chloroadenosine, PIA, or 5'-deoxyadenosine. PIA and 2-chloroadenosine were full agonists, whereas 5'-deoxyadenosine was a partial agonist, as in brain slices (17). In the presence of adenosine deaminase, 5'-AMP neither stimulated nor inhibited adenosine-sensitive adenylate cyclase (Fig. 2). ATP did not have any effect in either the presence or absence of adenosine deaminase, since the adenosine stimulation was apparent in the presence of 2 mM ATP. In intact brain tissues, 5'-AMP and ADP were as effective as adenosine in stimulating cyclic AMP accumulation (1). Furthermore, like adenosine, these agents inhibited the postsynaptic potential of olfactory cortex neurons (18). In fact, in both brain tissue and cell-free homogenates, 5'-AMP and ATP were partially hydrolyzed to adenosine, but in the cell-free assay this indirect effect of 5'-AMP and ATP could be eliminated by adding adenosine deaminase. 2'- and 3'-Deoxyadenosine are antagonists of the cyclic AMP accumulation induced by adenosine in brain tissue (2). In striatal homogenates, these compounds inhibited adenosine-sensitive adenylate cyclase noncompetitively (Fig. 3). In neuroblastoma cells, 2'-deoxyadenosine inhibits the cyclic AMP production induced by compounds such as biogenic amines and prostaglandins, which are not structurally related to adenosine (19). Our results confirm the existence of a specific adenosine receptor site involved in adenylate cyclase activation and rule out the possibility that the effect of adenosine on cyclic AMP accumulation in intact tissue may be due to its conversion to ATP in a pool accessible to the adenylate cyclase.

Von Hungen and Roberts (20) described $Ca^{2+}$-sensitive adenylate cyclase in the rat cerebral cortex. We found that rat striatal homogenates also contain a $Ca^{2+}$-sensitive adenylate cyclase (Figs. 5 and 7). Compared with the other adenylate cyclase activities, $Ca^{2+}$-sensitive adenylate cyclase activity was variable: 130 and 340 pmoles/min/mg in the experiments reported in Figs. 5 and 7, respectively. In five different experiments the activities were 270, 364, 340, 160, and 130 pmoles/min/mg. Such variations from one experiment to another

have often been reported for adenylate cyclase activities in cell-free preparations. Similar $Ca^{2+}$ concentrations stimulated both the adenylate cyclase and the phosphodiesterases present in rat striatal homogenates (data not shown). $Ca^{2+}$-induced stimulation of adenylate cyclase is probably mediated by the $Ca^{2+}$-binding protein described by Cheung (21) and Brostrom *et al.* (22), since in the presence of $Ca^{2+}$ this protein was shown to stimulate both 3',5'-nucleotide phosphodiesterases and adenylate cyclase.

Striatal homogenates contain specific adenylate cyclases sensitive to dopamine, *beta* adrenergic agonists (15), adenosine, and $Ca^{2+}$. We found that the activities of the dopamine-, adenosine-, and $Ca^{2+}$-sensitive adenylate cyclases were additive (Fig. 7). Adenosine did not modify the apparent affinity of dopamine for its specific receptor (Fig. 6).

The additive effect of the three agonists on the adenylate cyclase activity of rat striatal homogenates might suggest that their receptors are localized on different cell types, since no clear report exists on the additivity of hormone effects on one cell type. Furthermore, the dopamine- and *beta* adrenergic-sensitive adenylate cyclase activities in the frontal cortex of rat homogenates, which are also additive (23), have different topographical distributions (23).

Adenosine is specifically taken up (9, 10) and released (24) from synaptosomal preparations; our results show that adenosine is also able to interact with specific receptors coupled with an adenylate cyclase. Adenosine is thus a candidate for a purine neurotransmitter in the brain.

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