

ADENOSINE-SENSITIVE ADENYLATE CYCLASE IN RAT STRIATAL HOMOGENATES

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1. Introduction

There is a good deal of evidence suggesting that adenosine and its phosphorylated derivatives (adenosine mono-, di- and triphosphate) might act in the brain like a neurohumoral substances. These compounds can be taken up into brain slices [1,2] or synaptosomal fractions [3] through a specific uptake system. Furthermore they are released by electrical field stimulation and K^+ [2,4,5] such release being Ca^{2+} dependent [3]. Adenosine and its phosphorylated derivatives inhibit the firing of Purkinje [6] and olfactory cortex neurones [7]. Like other well-known neurotransmitters, adenosine derivatives induce the accumulation of adenosine 3',5'-monophosphate (cyclic AMP) in brain slices [1,7,11] and neuronal cell lines in culture [12,15]. Two mechanisms may be involved in this effect. Adenosine can be converted into ATP in a pool accessible to adenylate cyclase, but this is unlikely, since prevention of adenosine uptake by dipyrindamole does not inhibit cyclic AMP accumulation induced by adenosine [12]. In addition, 2 chloro-adenosine, which is not converted into ATP, increases cyclic AMP production [16]. Adenosine (and possibly its phosphorylated derivatives) could increase cyclic AMP production by acting extra cellularly on a specific membrane receptor coupled with an adenylate cyclase. The present report confirms this last hypothesis since we were able to demonstrate the presence of an adenosine-sensitive adenylate cyclase in striatum homogenates.

2. Methods

Male Charles River rats of the Sprague Dawley

strain (350–450 g) were killed by decapitation, their brain removed and the striata dissected at 4°C with glass manipulators. Tissues were homogenized (five strokes) using a Dounce homogenizer in 2 mM Tris–maleate, pH 7.2 and 2 mM EGTA (2 striata in 3 ml) at 4°C. Homogenates were filtered through a silk-screen (150 μ m pore diameter). Homogenate, 20 μ l, was then added to 60 μ l of a solution containing 41 mM Tris–maleate, pH 7.2, 1.6 mM $MgSO_4$, 0.42 mM ATP, 33 mM creatine phosphate, 0.33 mg/ml creatine kinase, 1.6×10^{-4} M papaverine and adenosine-deaminase (0.4 IU/ml) when necessary. After 2 min incubation at 30°C, 20 μ l of a solution containing 1–3 μ l [α - ^{32}P]ATP and 0.001 μ l cyclic-[3H]AMP as an internal recovery standard were added. The reaction was allowed to proceed for 5 min. The cyclic-[α - ^{32}P]AMP formed was isolated according to Salomon et al. [17].

3. Results and discussion

When the phosphodiesterases present in the homogenates were inhibited by papaverine, the addition of methylxanthines (theophylline, caffeine and isobutylmethylxanthine (IBMX) reduced adenylate cyclase activity by about 60% (fig.1). In this experiment, papaverine effectively blocked the phosphodiesterases since the addition of 1 mM unlabelled cyclic AMP, which should reduce the eventual hydrolysis of the labelled cyclic AMP formed, did not increase the amount of labelled cyclic AMP recovered (fig.1). Moreover external cyclic-[3H]AMP was added during incubation to correct the slight hydrolysis of cyclic-[^{32}P]AMP formed during the assay. In any case, the decrease in adenylate cyclase activity induced by methylxanthines cannot be related to the latter's

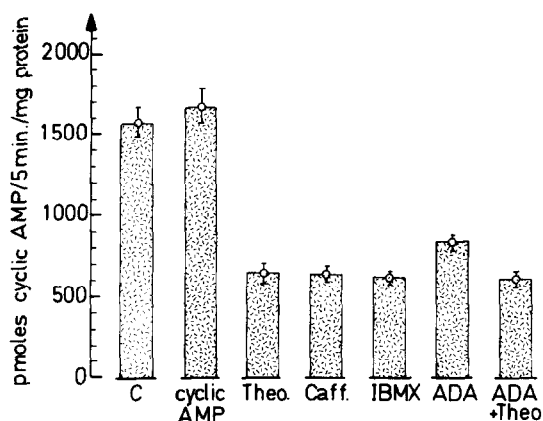


Fig. 1. Effect of cyclic AMP, methylxanthines and adenosine-deaminase on the adenylate cyclase activity of rat striatal homogenates. The concentrations of drugs were as follows: cyclic AMP 10^{-3} M, theophylline, (Theo) 6×10^{-4} M, caffeine (Caff) 5×10^{-3} M, isobutylmethylxanthine (IBMX) 10^{-3} M and adenosine-deaminase (ADA) 0.4 IU/ml. The protein concentration in the assay was 0.310 mg/ml. Each value is the mean \pm SE of three determinations.

known phosphodiesterase-blocking activity. Since methylxanthines prevent the increase in cyclic AMP accumulation induced by adenosine in brain slices [8], their inhibiting effect on the adenylate cyclase activity in our cell-free preparation might result from the blockade of an adenosine-sensitive adenylate cyclase stimulated by endogeneous adenosine. To test this hypothesis, adenosine-deaminase (ADA) was added for a preincubation period of 2 min and throughout the adenylate cyclase assay. As shown in fig. 1, ADA reduced control adenylate cyclase activity by 47%. Furthermore, the ADA and theophylline effects were not additive. These results confirm the presence of adenosine in the incubation medium and its stimulating effect on basal adenylate cyclase activity. The inhibition of the adenylate cyclase activity by theophylline, was concentration-dependent and was reversed by increasing concentrations of adenosine (fig. 2). In the absence of theophylline, adenosine did not significantly enhance cyclic AMP production (fig. 2). Thus, adenosine was present in the assay at a concentration leading to maximal stimulation of the adenosine-sensitive adenylate cyclase. In preliminary experiments, an adenosine concentration of $10 \pm 2.25 \times 10^{-6}$ M ($n = 4$) was found in striatal homogenates correspond-

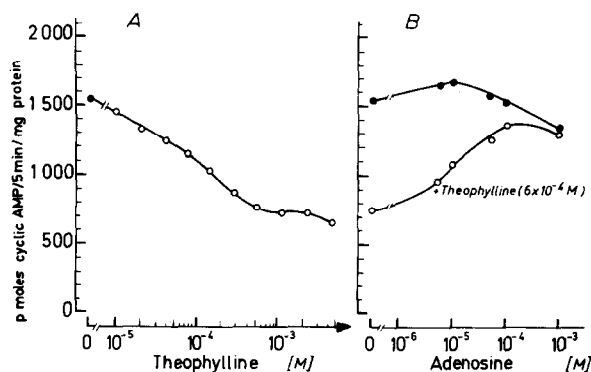


Fig. 2. Effect of theophylline and adenosine on adenylate cyclase activity. A, Dose-dependency of theophylline inhibition. B, Effect of adenosine on adenylate cyclase activity in the absence and presence of theophylline (6×10^{-4} M). The experiment was conducted in the absence of ADA. The protein concentration in the assay was 0.280 mg/ml. Each value is the mean of two determinations with 5% of the mean.

ing to a final concentration of 2×10^{-6} M in the assay. The slight inhibition of adenylate cyclase activity by high concentrations of adenosine ($> 10^{-5}$ M, fig. 2) was probably a result of the competition between adenosine and ATP for the catalytic site of the adenylate cyclase.

The effects of several adenosine analogs and derivatives on the adenosine-sensitive adenylate cyclase were evaluated under three conditions:

- (i) Control (presence of endogeneous adenosine).
- (ii) In the presence of ADA.
- (iii) In the combined presence of ADA and *N*₆-phenyl-isopropyl-adenosine (PIA), an adenosine analogue resistant to the deamination.

5'-Adenosine-monophosphate (5'-AMP) was neither an agonist nor an antagonist of the adenosine receptor (table 1). This contrasts with what was observed in brain slices [7,8,11]. A dephosphorylation of 5'-AMP might occur in intact tissues. Inosine, the product of adenosine-deaminase and adenine, the precursor of adenosine, did not stimulate or inhibit the adenosine-sensitive adenylate cyclase (table 1). 2'- and 3'-deoxy-adenosine reduced the stimulating effect of adenosine (see Control, table 1). However this effect was non-competitive with adenosine and was probably due to an action on the catalytic site of the enzyme (manuscript in preparation). 2-chloro-adenosine and PIA,

Table 1
Specificity of adenosine-sensitive adenylylase

Addition	Adenylylase activities (cyclic AMP pmol/5 min/mg protein)			
	Control	ADA	ADA + PIA	Deaminated by ADA
None	1767	1093	1770	
Adenine	1697	976	1697	—
Adenosine	1720	nd	nd	+
2'-Deoxyadenosine	1441	1116	1767	+
3'-Deoxyadenosine	1395	1069	1860	+
5'-Deoxyadenosine	1511	1418	1395	—
2-chloro-adenosine	1786	1802	1848	—
5'-AMP	1744	976	1697	—
Inosine	1674	976	1697	—

Drug concentrations were 10^{-4} M except for 5'-AMP and PIA which were at 10^{-5} M. The protein concentration was 0.43 mg/ml. Each value is the mean of two determinations which were within 5% of the mean.

two compounds not deaminated by ADA, were potent agonists (table 1 and fig.3). Finally, as in brain slices [11], 5'-deoxyadenosine was found to be a partial

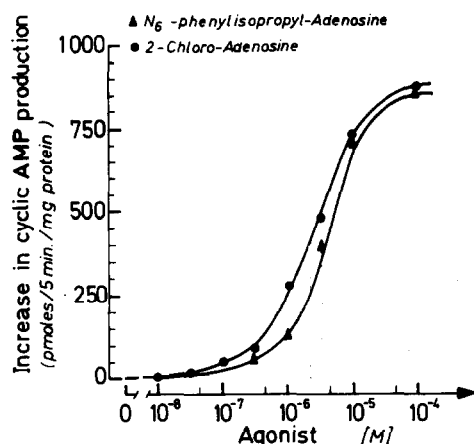


Fig.3. Effect of 2-chloro-adenosine and N_6 -phenyl-isopropyl-adenosine (PIA) on adenosine-sensitive adenylylase activity. The experiment was conducted in the presence of adenosine-deaminase (0.4 IU/ml). These two adenosine analogues were chosen because of their resistance to deamination. In the presence of ADA and absence of an agonist, the adenylylase activity was 877 ± 14 pmol/5 min/mg protein ($n = 4$). The apparent affinities of 2-chloro-adenosine and N_6 -phenyl-isopropyl-adenosine for the adenosine receptor site were 1.2×10^{-6} M and 3.2×10^{-6} M respectively. The protein concentration was 0.290 mg/ml. Each value is the mean of two determinations which were within 5% of the mean.

agonist of the adenosine-sensitive adenylylase (table 1). Whereas adenosine is known to stimulate adenylylase in a cell-free preparation from several cultured nervous cell lines [13,14], fibroblasts and bone cells [18], the foregoing results provide, to our knowledge, the first indication of an adenosine sensitive adenylylase in a cell-free preparation from central nervous system.

The presence of an adenosine-sensitive adenylylase in a cell-free preparation of the brain confirms the assumption that, in brain slices, adenosine-induced cyclic AMP formation is mediated by the interaction of adenosine with a specific receptor coupled with an adenylylase. This adenylylase system could be of further help in studying the possible role of adenosine in neurotransmission processes. The adenosine-sensitive adenylylase in cell-free preparation is exclusively stimulated by adenosine, which contrasts with what was observed in brain slices, where phosphorylated derivatives of adenosine acted as agonists [7,8]. Adenosine is therefore the best candidate to be the purine neurotransmitter in the central nervous system.

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