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SHORT COMMUNICATIONS

Adenosine Receptor-Mediated Inhibition of Rat Cerebral Cortical Adenylate Cyclase by a GTP-Dependent Process

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SUMMARY

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Adenosine analogues inhibit rat cerebral cortical adenylate cyclase by a receptor-mediated GTP-dependent process. N^6-Phenylisopropyladenosine and other analogues of adenosine are potent inhibitors (K_i values of 10–100 nM) of adenylate cyclase in a particulate preparation of rat cerebral cortex. The detection of such inhibitory effects is facilitated by the use of a deoxy ATP assay system. The inhibition is strictly dependent on GTP, is amplified by Na^+ ions, and is antagonized by methylxanthines.

INTRODUCTION

A considerable body of evidence indicates that adenosine, generated endogenously in cerebral tissues, acts through "purinergic receptors" to depress neuronal firing (1, 2). It has been even suggested that the actions of tricyclic antidepressants and opiates might be mediated by a secondary release of adenosine (3). Sattin and Rall (4) and Shimizu and Daly (5) demonstrated that adenosine increases cyclic AMP[1] production in brain slices by acting at a site which has the characteristics of the cell surface adenosine receptor subsequently identified in a large number of tissues (6). Such findings led to the proposal (7) that the antidepressant effects of adenosine were mediated by adenylate cyclase (EC 4.6.1.1). However, it is by no means certain that the physiological effects of adenosine are mediated by increased cyclic AMP production, since a distinct lack of correlation has been observed between cyclic AMP levels and the neurogenic effects elicited by the nucleoside (8). On the other hand, it is entirely possible that at least some of the actions of adenosine are mediated by inhibition of cyclic AMP production. There are reports of receptor-mediated inhibition (9) and stimulation (10, 11) of cyclic AMP production in cell lines derived from brain.

Recently we have shown (12) that detection of adenosine receptors linked to adenylate cyclase is greatly facilitated by reducing the concentration of adenosine in the assay medium by substituting dATP for ATP as substrate. In addition, we have shown that GTP is essential for the expression of both the stimulatory and the inhibitory effects of adenosine acting through surface membrane receptors (6, 13). Here we show that the adenylate cyclase system(s) in the brain cortex is sensitive to an inhibitory effect of adenosine and that the receptor involved has several features in common with adenosine receptors found elsewhere.

Rat brain cortical membranes were prepared as previously described (14). Briefly, on excision of the cortex from freshly decapitated rats, the material was sliced and incubated at 37°C for 1 h in Krebs–Ringer bicarbonate buffer under 95% O_2/5% CO_2. The tissue slices were then transferred to ice-cold 50 mM Tris–HCl, pH 7.4, homogenized in a tight-fitting Dounce homogenizer (Model 29), and centrifuged (3000*g*, 10 min, 4°C). The pellet was resuspended and washed by centrifugation three times in 50 mM Tris–HCl, pH 7.4. Adenylate cyclase was assayed as described previously (12) in a medium containing 0.1 mM [α-^{32}P]dATP (1 μCi), 0.1 mM cyclic dAMP, 30 mM Tris–HCl, pH 7.4, 5 mM $MgCl_2$, 5 mM creatine phosphate, 25 U/ml creatine phosphokinase, and 0.04% bovine serum albumin. Assays were normally performed in a volume of 100 μl containing 5–10 μg of membrane protein for 15–20 min at 24°C. Adenosine deaminase (2.5 U/ml) was included in the majority of experiments as a precaution against the possibility of endogenous adenosine in the brain preparations. However, very similar results were obtained in a number of experiments (not shown) where the enzyme was omitted from the medium. Activity was linear at 24°C for at least 30 min. Cyclic dAMP was eluted by the procedure previously described (12). Assays were performed in triplicate and results quoted are typical of three to five experiments covering at least three membrane preparations.

[1] Abbreviations used: cyclic AMP, cyclic 3′,5′-adenosine monophosphate; PIA, N^6-phenylisopropyladenosine.

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Figure 1 shows that, in the absence of Na^+ ions, GTP, at concentrations above 40 nM, evokes a small inhibition of adenylate cyclase activity in membranes from brain cortex. This inhibitory phase was accentuated in the presence of phenylisopropyladenosine (PIA), an analogue of adenosine previously shown to be an agonist at adenosine receptors in a number of cells (15, 16). Studies of the adipocyte adenylate cyclase system, which also exhibits GTP inhibition that is potentiated by PIA (13), have shown that Na^+ amplifies the inhibitory effects of PIA and GTP by opposing the inhibitory effects of GTP alone (15; Londos and Cooper, in preparation). In the case of the cortex membranes, sodium ion (80 mM) largely eliminated the inhibitory effects of GTP, in the absence of PIA (Fig. 1), which resulted in a slight amplification of the inhibitory effects seen in the presence of PIA. The latter point is demonstrated in Fig. 2, where the concentration dependence of the NaCl effect is examined. The activity increases in proportion to the salt concentration, in the absence of PIA. However, in the presence of the nucleoside, the activity is unchanged by alterations in the salt concentration.

The inhibitory effects of PIA were compared with those given by other adenosine analogues (Fig. 3). The K_i values obtained in the presence of 4 μM GTP were 10 nM for both PIA and N^6-cyclohexyladenosine and 100 nM with 5′-*N*-ethylcarboxamide adenosine. These K_i values are to be compared with the far greater K_a values for adenosine analogues involved in the stimulation of adenylate cyclase (normally in the range of 1–10 μM; 6, 15). In the face of these results it may be readily appreciated that the presence of inhibitory sites of such sensitivity may have gone unnoticed using conventional ATP assay systems, since a very low conversion of substrate to adenosine would result in total expression of the inhibitory effects. In fact, when we attempted to detect inhibition by PIA using a conventional ATP assay method, in the presence of adenosine deaminase (2–5 U/ml), we could detect only 10% effects under conditions which led to 35% inhibition using dATP as substrate (results not shown).

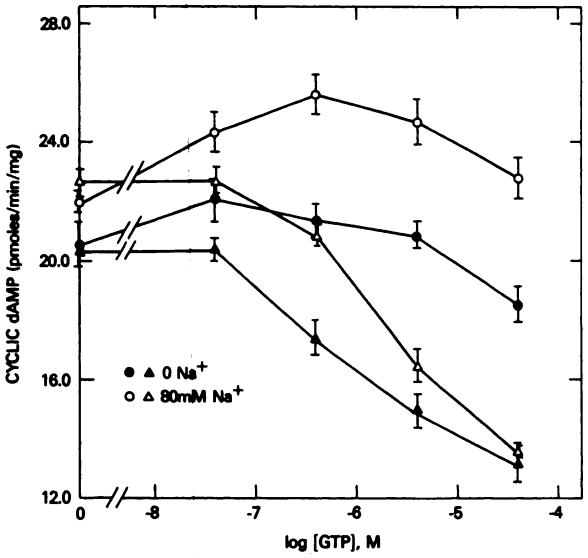

FIG. 1. *Cerebral cortical adenylate cyclase activity as a function of GTP concentration*

Cortical particulate preparations were assayed as described in the text with GTP at the indicated concentrations, in the absence (●, ▲) or presence (○, △) of 80 mM NaCl with (△, ▲) and without (○, ●) 10 μM PIA. Means and standard deviations of triplicate determinations from a single typical experiment are shown.

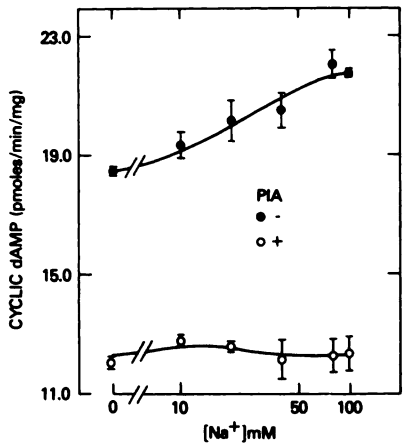

FIG. 2. *Cerebral cortical adenylate cyclase activity in relation to NaCl concentration*

Adenylate cyclase activity (assayed as described in the text) was determined with 40 μM GTP in the absence (●) and presence (○) of 10 μM PIA and the indicated NaCl concentrations.

The same relative potency of these adenosine analogues has been observed with the inhibitory adenosine receptor (R_i type) in rat adipocytes and is opposite to the order of potency of these analogues as stimulators of adenylate cyclase activity; the latter occur through adenosine receptors (R_a type) that are pharmacologically different from the inhibitory adenosine receptors (15, 17).

Figure 4 illustrates antagonism of the inhibitory effects of PIA by 3-methyl-1-isobutylxanthine. The K_i for the methylxanthine (from Schild plots of the data) was 0.45 μM. This value is two orders of magnitude lower than the

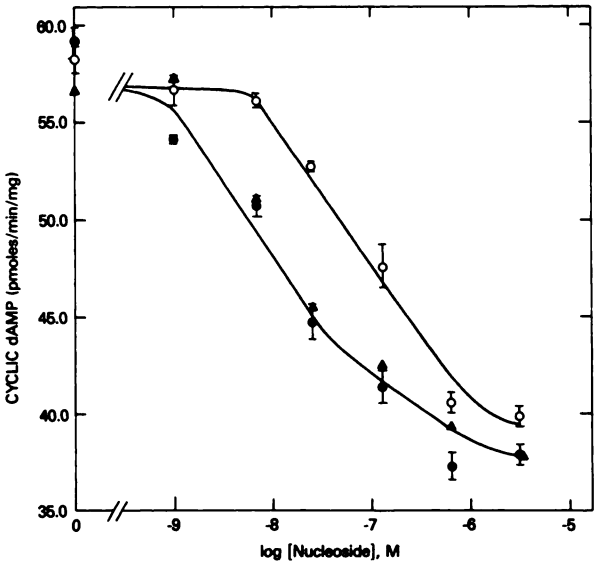

FIG. 3. *Inhibition of cerebral cortex adenylate cyclase by adenosine analogues*

Adenylate cyclase activity was measured in the presence of 4 μM GTP, 80 mM NaCl, and the indicated concentrations of PIA (●), N^6-cyclohexyladenosine (▲) and 5′-*N*-ethylcarboxamide adenosine (○).

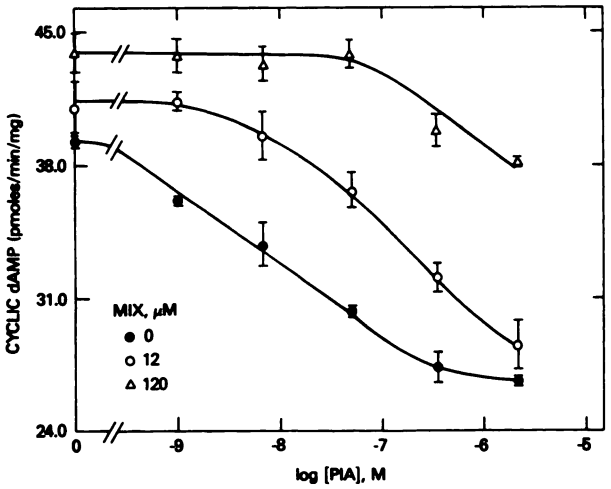

FIG. 4. *Antagonism of PIA inhibition by methylisobutylxanthine*
Adenylate cyclase activity was determined in the presence of 4 μM GTP, 80 mM NaCl, and the indicated concentrations of PIA with either 0 (●), 12 μM (○), or 120 μM (△) 3-methyl-1-isobutylxanthine.

K_i for inhibition of cyclic nucleotide phosphodiesterase activity by methylxanthines (18). The low value is in keeping with the known potent inhibitory effects of methylxanthines on adenosine receptors and is indistinguishable from the K_i reported for 1-methyl-3-isobutylxanthine on the inhibitory adenosine receptor in the adipocyte (12, 13).

The above findings indicate that the rat brain cortex contains an adenylate cyclase system to which an adenosine receptor is functionally linked. This adenosine receptor displays several properties in common with the adenosine receptor in the rat adipocyte adenylate cyclase system. In both cases, purine-modified adenosine analogues inhibit adenylate cyclase activity, the effects are GTP dependent, methylxanthines competitively inhibit the agonists, and sodium ion appears to modulate the adenosine response.[2] One important aspect of the present findings is that adenosine receptors have hitherto been shown only to mediate activation of adenylate cyclase in membranes of neuronal origin (10, 11), and the latter systems also exhibit a GTP requirement.[3] The finding of a GTP-inhibitory process promoted by adenosine suggests that adenylate cyclase in the cortex may be under two independent control processes each governed by different receptors and GTP-regulatory proteins, one set of receptors and nucleotide regulatory proteins governing stimulation of adenylate cyclase and the other set inhibition of the enzyme. This proposal is based in part on the demonstration of separable stimulatory and inhibitory GTP-mediated processes in the adipocyte which function to amplify the effect of stimulatory and inhibitory ligands, respectively (19). It is also based on the findings that opiates, catecholamines, and cholinergic agents (20–22) as well as adenosine may cause inhibition of adenylate cyclase which is GTP dependent and Na^+ regulated as discussed in recent reviews (15, 23). Thus, the finding that adenosine inhibits adenylate cyclase in brain membranes with properties similar to those of other known neurotransmitters puts the present findings in a larger perspective of how such agents act on adenylate cyclase systems. It remains to be seen whether the inhibitory effects of adenosine seen on cortex membranes have any correlation with the physiological effects of this compound on brain cortex metabolism.

[2] The receptor-mediated (R-site) adenosine analogue inhibition, described here, is not to be confused with P-site inhibition, which has been encountered in every tissue examined, including brain (6, 24). Such inhibition is yielded selectively by ribose-modified adenosine analogues, such as 2′,5′-dideoxyadenosine, with far higher K_i values (in the range of 5–25 μM); it is not dependent on GTP and is not reversed by methylxanthines (6).

[3] Since calmodulin-stimulated adenylate cyclase is considered to comprise a significant proportion of cortical enzyme activity (25), preliminary attempts were made to evaluate the role, if any, of this component on our observations. Experiments involving either prewashing of membranes with 2 mM EGTA and 1 M NaCl, a procedure reported to remove bound calmodulin (26), or inclusion of up to 1 mM EGTA in the assay evoked a 60% inhibition of enzyme activity, but no alteration in the inhibitory effects of PIA (results not shown).

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