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Specificity of Adenosine Inhibition of cAMP-Induced Responses in *Dictyostelium* Resembles That of the P Site of Higher Organisms

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Adenosine acts as a cyclic AMP antagonist in *Dictyostelium discoideum*. It inhibits the binding of cyclic AMP to cell surface receptors and the induction of postaggregative differentiation by cyclic AMP. We investigated the nucleoside specificity and dose dependency of both inhibitory effects of adenosine. It was found that adenosine inhibits cyclic AMP binding and cyclic-AMP-induced differentiation with a K_i of about 300 μM. Alterations in the purine moiety of adenosine generally decrease the inhibitory effect of the molecule, whereas alterations in the ribose moiety are tolerated and in most cases even increase the inhibitory effect of the molecule on both cyclic AMP binding and differentiation induction. A strong correlation ($r = 0.996$, $P < 0.01\%$) between the specificities for adenosine derivatives of these two inhibitory processes is demonstrated. The nucleoside specificity for the inhibition of cyclic AMP action in *D. discoideum* resembles that of the P site of higher organisms. In contrast to effects mediated by the P site of higher organisms, the effects of adenosine mediated by the *Dictyostelium* receptor cannot be prevented by inhibiting adenosine uptake; this makes it very likely that the adenosine receptor, which is involved in the effects of adenosine on cyclic AMP binding and differentiation induction, is located at the cell surface. © 1986 Academic Press, Inc.

INTRODUCTION

Vegetative cells of *Dictyostelium discoideum* feed on bacteria and enter a process of development and differentiation upon starvation. One of the first steps in this process is the acquisition of aggregation competence. Some cells autonomously start to secrete pulses of cyclic AMP, surrounding cells detect cyclic AMP by means of cell surface receptors and respond to the signal by chemotaxis to the cyclic AMP source and by secreting a cyclic AMP pulse themselves. The original signal is thus efficiently relayed through the cell population and the cells aggregate to form multicellular structures called slugs. In these slugs two cell types can be distinguished: (1) cells destined to form stalk cells, which retain a biochemical and functional resemblance to aggregation competent cells, and (2) prespore cells, which anticipate ultimate spore differentiation by the early expression of spore-specific genes. Ultimately slugs culminate to form a fruiting structure consisting of a globular mass of spores supported by a column of stalk cells (for review, see Loomis, 1975 and 1982).

Besides functioning as a chemoattractant, extracellular cyclic AMP also functions as a morphogen (Town and Gross, 1978; Chisholm *et al.*, 1984); Submillimolar concentrations of cyclic AMP can induce prespore specific gene expression through interaction with the cell surface receptors (Schaap and Van Driel, 1985).

One of the physiological degradation products of cyclic AMP, adenosine, has been shown to inhibit several cyclic-AMP-mediated responses during this developmental process. Adenosine inhibits autonomous cyclic AMP signaling (Newell and Ross, 1982), cyclic AMP relay (Brenner and Thoms, 1984; Theibert and Devreotes, 1984), chemotaxis, and cyclic-AMP-induced cyclic GMP response (Van Haastert, 1983). Recently, evidence was provided that interactions between adenosine and the cyclic AMP signaling system play important roles in the formation of the prespore/prestalk pattern of *Dictyostelium* slugs; adenosine prevents the conversion from prestalk to prespore cells (Weijer and Durston, 1985) and inhibits the induction of prespore differentiation by cyclic AMP (Schaap and Wang, 1986). It was hypothesized that production of adenosine by hydrolysis of cyclic AMP in the anterior region of the slugs inhibits prespore differentiation and is thus responsible for the establishment of the prestalk/prespore pattern (Schaap and Wang, 1986). This hypothesis is supported by the observation that the cyclic AMP hydrolyzing enzymes, cyclic AMP-phosphodiesterase and 5'AMP-nucleotidase, are preferentially active at slug anteriors (Armant *et al.*, 1980; Brown and Rutherford, 1980; Tsang and Bradbury, 1981; Schaap and Spek, 1984) and by the fact that removal of endogenous adenosine from slugs results in prespore differentiation in slug anteriors (Schaap and Wang, 1986). At least some, but possibly all, effects of adenosine on cyclic-AMP-induced responses are presumed to result from the inhibitory effect of adenosine

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on the binding of cyclic AMP to its cell surface receptor (Newell and Ross, 1982; Van Haastert, 1983; Theibert and Devreotes, 1984).

Dictyostelium cells were found to have two types of [^{3}H]adenosine binding sites; the β receptor (K_d = 350 μM; 8 $\times$ 10^6 sites/cell), and the α receptor (K_d = 0.8 μM; 3.5 $\times$ 10^4 sites/cell) (Newell, 1982; Van Haastert, 1983). Due to the fact that millimolar concentrations of adenosine are required to inhibit cyclic-AMP-induced responses, it is likely that these effects of adenosine are mediated through the β receptor.

In higher organisms three types of adenosine binding sites can be distinguished, all of which affect the receptor-mediated adenylate cyclase activation (for reviews, Wolff *et al.*, 1981; Daly, 1985): (1) The intracellularly located P site ($K_d > 10^{-5}$ M), which probably inhibits activation of adenylate cyclase by direct interaction with the catalytic subunit of this enzyme. (2) The R_a site or A_2 receptor (K_d in the micromolar range), which is located at the cell surface and stimulates the activation of adenylate cyclase presumably by interacting with the stimulatory GTP-binding protein N_s. (3) The R_i site or A_1 receptor (K_d in the nanomolar range), which inhibits the activation of adenylate cyclase and is thought to act on the inhibitory GTP-binding protein N_i.

P and R sites can be distinguished by their specificity and affinity (Londos and Wolff, 1977). P sites require an intact purine moiety, whereas R sites require an intact ribose moiety. R_a and R_i sites can be distinguished by their differential affinity for adenosine, (-)N6-R-(2-phenylisopropyl)adenosine (PIA), and 5'-*N*-carboxamide adenosine (NECA) (Londos *et al.*, 1980).

In this study, we have analyzed whether the effect of adenosine on cyclic AMP binding in *Dictyostelium* is mediated through a P or R type interaction. This was done by investigating the inhibition of cyclic AMP binding by a series of adenosine derivatives, modified at different sites in the purine or ribose moiety, on cyclic AMP binding. It is shown that the specificity of the *Dictyostelium* adenosine receptor is similar to that of the P site of higher organisms, but is different in that it is located at the cell surface and not intracellularly. Furthermore, the nucleoside specificity for inhibition of cyclic AMP binding closely resembles the specificity for inhibition of cyclic-AMP-induced responses, such as the induction of differentiation.

MATERIALS AND METHODS

Materials. [2,8-^{3}H]cyclic AMP (1.54 TBq/mmole), [2,5',8-^{3}H]adenosine (1.5 TBq/mmole), and L-[1-^{14}C]ornithine (2.11 GBq/mmole) were from The Radiochemical Centre, Amersham, U.K. Adenosine, adenosine 5'-triphosphate, and PIA were from Boehringer-Mannheim, F.R.G. 3-deazaadenosine was from the Southern Research Institute, Birmingham, Alabama, and NECA was a generous gift from the Byk Gulden Lomberg Chemische Fabrik GmbH, Konstanz, F.R.G. All other adenosine derivatives and *S*-*p*-nitrobenzyl-6-thioinosine were from Sigma, St. Louis, Missouri. Adenosine 3':5'-monophosphorothioate S_p isomer ((S_p)-cyclic AMPS) was kindly provided by Dr. J. Baraniak and Dr. W. Stec, Polish Academy of Science, Lodz, Poland (Baraniak *et al.*, 1979).

Organisms and culture conditions. D. discoideum strain NC4(H) was grown in association with *Escherichia coli* 281 on a solid medium containing 3.3 g peptone, 3.3 g glucose, 4.5 g KH_2PO_4, 1.5 g $Na_2HPO_4 \cdot 2H_2O$, and 15 g agar per liter. Vegetative cells were harvested with 10 mM phosphate buffer, pH 6.5, and freed from bacteria by repeated centrifugation.

Cyclic AMP binding assay. [2,8-^{3}H]cyclic AMP binding was assayed at 1 nM by means of the ammonium sulfate stabilization assay as described by Van Haastert and Kien (1983).

Adenosine uptake assay. Vegetative cells were harvested and then starved for 5 hr in 10 mM phosphate buffer, pH 6.5, at a density of 10^7 cells/ml. The uptake of [2,5',8-^{3}H]adenosine was measured by incubating 100 μl cells with 10^{-8} M [2,5',8-^{3}H]adenosine and different concentrations of unlabeled adenosine or inosine for 1 hr at room temperature. Subsequently, unlabeled adenosine (10^{-2} M final concentration) was added to displace any adenosine bound to the cell surface, and 1 min later the cells were centrifuged through 12% polyethyleneglycol (PEG) in 10 mM phosphate buffer, pH 6.5, during 2 min at 10,000*g*. After removal of the supernatant, the pellet was dissolved in 110 μl 1 M acetic acid. The radioactivity of 100 μl was determined.

Assay for measuring ornithine decarboxylase induction. Vegetative cells were freed from bacteria and distributed on nonnutrient agar plates (Ø 10 cm) at a density of 2.5 $\times$ 10^8 cells/plate and then incubated for 16 hr at 6°C to induce full aggregation competence (Konijn, 1970; Van Lookeren Campagne and Löwik, 1985). Cells were then collected and resuspended to 1.25 $\times$ 10^7 cells/ml. Forty microliters of cell suspension were incubated for 5 hr at 21°C with 5 μl (S_p)-cyclic AMPS and 5 μl adenosine derivatives, in 1.5-ml Eppendorf tubes, covered with moist tissue paper to prevent excess evaporation. During the incubation period, tubes were shaken on an Eppendorf shaker type 5432, alternately for 1 sec with 2-sec intervals. Ornithine decarboxylase (L-ornithine carboxylyase, EC 4.1.1.17) activity was assayed directly using a modification of the assay previously described (Van Lookeren Campagne and Löwik, 1985). The caps of the tubes were equipped with a filter paper drenched in 10 μl of 50% KOH in water:glycerol (1:1). Five microliters

of a 1 mM, 5 μCi/ml solution of L-[1-^{14}C]ornithine was added to the cell suspension, the tubes were immediately closed and incubated for a further 45 min at 21°C. The reaction was stopped by adding 20 μl of 18% perchloric acid. After shaking for a further half hour, the caps were removed and were placed in scintillation vials. Filters were eluted with 0.5 ml water for 5 min on a rotary shaker and subsequently 4.5 ml scintillation fluid was added and the radioactivity of the vials was determined.

RESULTS AND DISCUSSION

Inhibition of Cyclic AMP Binding by Adenosine

Nucleotide specificity of the inhibitory effect of adenosine on the binding of [^{3}H]cyclic AMP to cell surface receptors was studied with 28 derivatives of adenosine (Table 1, Figs. 1 and 2). As is evident from these results,

TABLE 1
INHIBITION OF [^{3}H]CYCLIC AMP BINDING BY ADENOSINE AND ITS DERIVATIVES

No.	Derivative	IC_{50}[a]	$\delta\Delta G$[b]
1	Adenosine	296	0.0
2	Adenosine-N1-oxide	>100,000	>13.2
3	2-Chloroadenosine	20	−6.1
4	3-Deazaadenosine	11,000	8.2
5	Purineriboside	10,000	8.0
6	6-Chloropurineriboside	100	−2.5
7	N6-methyladenosine	710	2.0
8	N6-dimethyladenosine	1,300	3.4
9	Zeatine riboside	1,550	3.8
10	PIA	10,000	8.0
11	Inosine	>100,000	>13.2
12	7-Deazaadenosine	10,000	8.0
13	8-Bromoadenosine	750	2.1
14	Guanosine	100,000	13.2
15	Xanthosine	4,000	5.9
16	Adenine	2,600	4.9
17	2′-Deoxyadenosine	96	−2.6
18	2′-O-Methyladenosine	11	−7.5
19	Adenine-9-D-arabinofuranoside	58	−3.7
20	3′-Deoxyadenosine	86	−2.8
21	3′-O-Methyladenosine	170	−1.3
22	5′-Methylthioadenosine	90	−2.7
23	NECA	10	−7.7
24	2′, 3′-isopropylidenadenosine	13	−7.1
25	Adenosine 2′-monophosphate	720	2.1
26	Adenosine 3′-monophosphate	1,100	3.0
27	Adenosine 5′-monophosphate	>100,000	>13.2
28	Adenosine 5′-triphosphate	100,000	13.2

[a] IC_{50}s were determined from concentration dependency curves of the adenosine derivatives for the inhibition of 10^{-9} M [^{3}H]cyclic AMP binding, measured as described under Material and Methods.

[b] $\delta\Delta G$ values are used as a measure for the potency of individual derivatives relative to adenosine. These values are derived from the equation: $\delta\Delta G = -RT \ln(IC_{50}\ \text{derivative}/IC_{50}\ \text{adenosine})$.

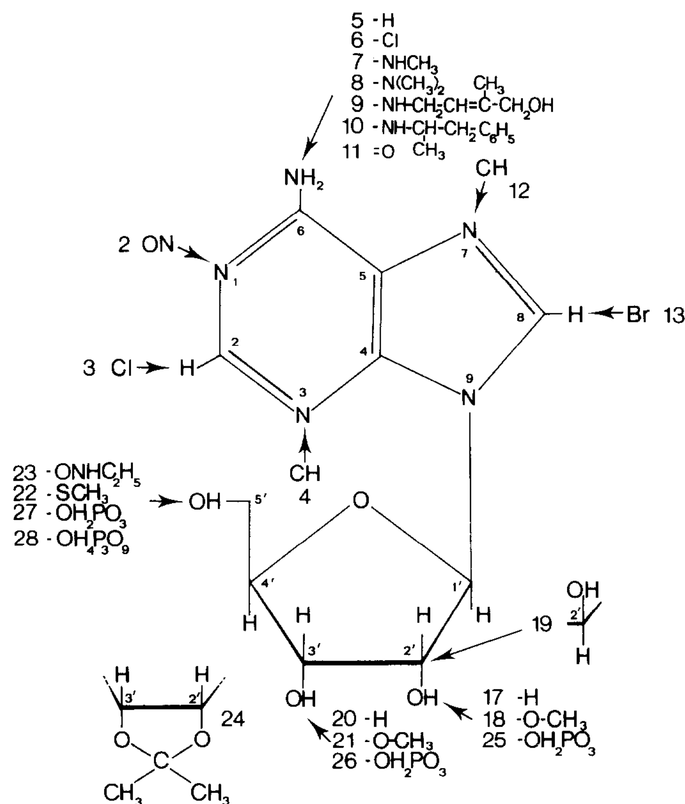

FIG. 1. Structures of adenosine derivatives.

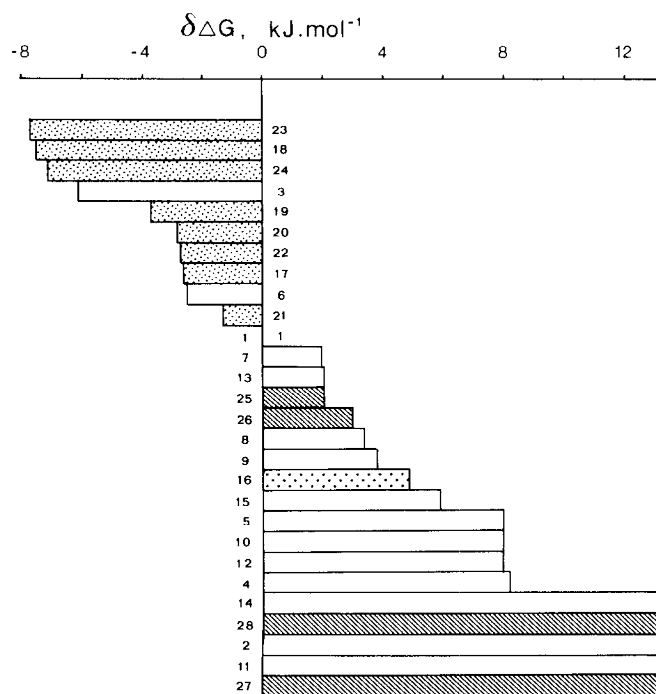

FIG. 2. Relative potencies of adenosine derivatives as inhibitors of cyclic AMP binding. Graphic representation of the $\delta\Delta G$ values from Table 1 arranged in order of potency. Dotted bars represent derivatives with altered ribose moiety, excluding the phosphorylated derivatives, which are represented by striped bars. Regularly dotted bars represent derivatives modified in both the ribose and the purine moieties, and open bars represent derivatives with altered purine moieties.

modifications in the ribose moiety of adenosine (derivatives 16–28; striped and dotted bars in Fig. 2) increase the effectiveness of the molecule for inhibiting cyclic AMP binding, with the exception of the phosphorylated derivatives (derivatives 25–28; striped bars in Fig. 2). Adenine (derivative 16; regularly dotted bar) has 10-fold lower activity than adenosine. Modifications in the purine moiety reduce the effectiveness of the molecule to inhibit cyclic AMP binding, the exceptions being: 2-chloroadenosine and 6-chloropurine riboside.

This type of specificity and affinity range are similar to the specificity and K_d of the P site of higher organisms (Londos and Wolff, 1977; Wolff *et al.*, 1981). The only exceptions are: (a) NECA, which binds very poorly to the P site (Londos *et al.*, 1980), but inhibits [^{3}H]cyclic AMP binding at 30-fold lower concentrations than adenosine, and (b) 5'-methylthioadenosine, which is also inactive at the P site (Daly, 1982), but inhibits [^{3}H]cyclic AMP binding at 3.3-fold lower concentrations than adenosine. R-site specificity is very different, in that all modifications in the ribose moiety, with exception of the 5'-carboxamides, cause a substantial reduction in affinity, whereas modifications at for example the N^6 position can cause an increase in affinity (Bruns, 1980; Premont *et al.*, 1979; Daly, 1985).

Effects of Adenosine on Cyclic AMP-Induced Responses

Previously, it has been shown that a correlation exists between the inhibition by adenosine of cyclic AMP binding and the inhibition of cyclic AMP relay, chemotaxis and the cyclic GMP response, when the effects of 2-chloroadenosine, 2'-deoxyadenosine, 2'-*O*-methyladenosine, and PIA were compared (Theibert and Devreotes, 1984; Van Haastert, 1983; data not shown), suggesting that the inhibitory effect of adenosine on the relay response is due to inhibition of cyclic AMP binding by adenosine. A similar correlation may exist between the inhibition of cyclic AMP binding and the inhibition of cyclic-AMP-induced differentiation by adenosine. We therefore compared the nucleoside specificity of these two processes.

As a marker for postaggregative differentiation, ornithine decarboxylase activity was chosen because the assay for this enzyme is sensitive, reproducible and accurate (Van Lookeren Campagne and Lowik, 1985). Ornithine decarboxylase, as well as other markers of postaggregative differentiation, can be induced in aggregation competent cells, shaken in phosphate buffer, by millimolar concentrations of cyclic AMP (Schaap and Van Driel, 1985) and adenosine can inhibit this induction in a competitive manner with an apparent K_i of about 200 μM (Schaap and Wang, 1986). The nonhydrolyzable cyclic AMP analog (S_p)-cyclic AMPS was used as the stimulus instead of cyclic AMP to avoid undue accumulation of adenosine through the hydrolysis of cyclic AMP. The induction of ornithine decarboxylase by various concentrations of (S_p)-cyclic AMPS is shown in Fig. 3. As is evident from these results, half-maximal enzyme induction by (S_p)-cyclic AMPS occurs at about 3 μM, which coincides well with the affinity of (S_p)-cyclic AMPS for the cyclic AMP receptor (Van Haastert, 1983). This indicates that the requirement of high concentrations of cyclic AMP for differentiation induction is very likely due to the high phosphodiesterase activity and possibly accumulation of adenosine in the suspension.

The inhibition of ornithine decarboxylase induction by adenosine and several adenosine derivatives is presented in Fig. 4. Low concentrations of (S_p)-cyclic AMPS were employed, which gave about 20% of the maximal induction. This was done to reduce the concentrations of adenosine derivatives required for effective inhibition, since most derivatives are not soluble at concentrations above 10 mM. As shown in Fig. 4, the order of potency of the analogs tested is NECA > 2'-*O*-methyladenosine > 5'-methylthioadenosine > 2'-deoxyadenosine > adenosine > PIA > guanosine > inosine.

The relative potencies of the inhibitory effects of the different adenosine analogs on cyclic AMP binding and ornithine decarboxylase induction can be normalized by calculating the $\delta\Delta G$ values in kilojoules per mole (where $\delta\Delta G = -RT \ln \mathrm{IC}_{50}\ \mathrm{derivative}/\mathrm{IC}_{50}\ \mathrm{adenosine}$; Jastorff *et al.* (1979)). When the $\delta\Delta G$ values of these two inhibitory effects of adenosine were plotted against each other, a straight line was obtained with a slope of 1.53

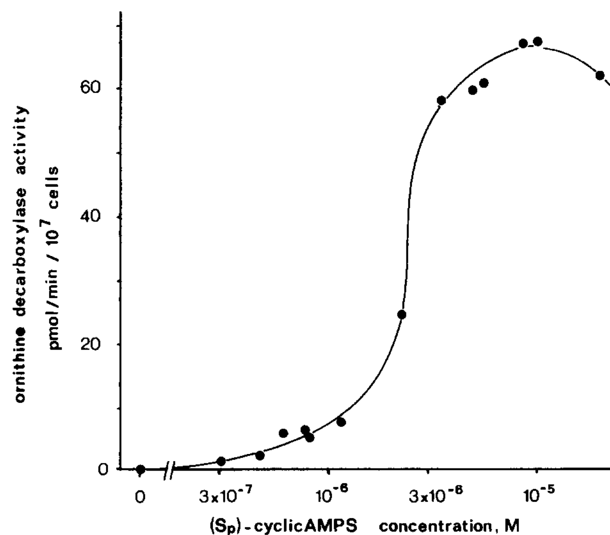

FIG. 3. Induction of ornithine decarboxylase activity by (S_p)-cyclic AMPs in aggregation competent cells. Ornithine decarboxylase induction was measured *in vivo* as described under Materials and Methods with different concentrations of (S_p)-cyclic AMPS. Data shown are the means of three experiments in triplicate.

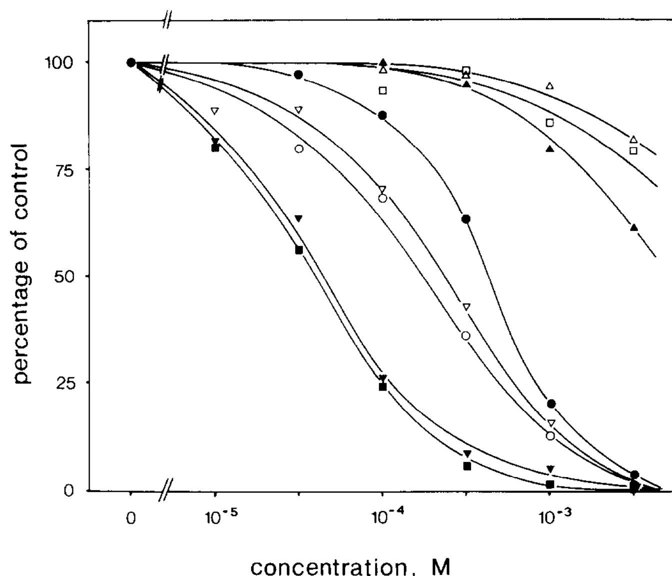

FIG. 4. Effect of several adenosine derivatives on the induction of ornithine decarboxylase. Aggregation competent cells were incubated for 5 hr with 2×10^{-6} *M* (S_p)-cyclic AMPs and various concentrations of different adenosine derivatives. Subsequently ornithine decarboxylase activity was measured as described under Materials and Methods. ●, Adenosine; ▲, PIA; △, inosine; □, guanosine; ▽, 2′-deoxyadenosine; ▼, 2′-*O*-methyladenosine; ○, 5′-methylthioadenosine; ■, NECA. Results shown are the means of at least three experiments in triplicate.

and a correlation coefficient of 0.996, $P < 0.01\%, n = 6$ (see Fig. 5). The relatively high slope implies that the inhibitory effect of adenosine on differentiation induction is less sensitive to modification of the adenosine molecule than the inhibitory effect of adenosine on cyclic AMP binding. The reason of this phenomenon is unknown. However, the high correlation coefficient indicates that both responses react similarly to the different types of modification. Thus it appears that the effects of adenosine on differentiation induction are mediated through a P-site interaction and that the inhibition of cyclic AMP binding by adenosine is very likely the cause of the inhibition of differentiation induction.

We considered whether the inhibitory effects of adenosine result from direct competition of adenosine with cyclic AMP for the cyclic AMP receptor. However, Fig. 6 demonstrates that similar modifications in the cyclic AMP and adenosine molecule affect cyclic AMP binding and inhibition of cyclic AMP binding by adenosine in an essentially different manner. It is thus unlikely that adenosine competes with cyclic AMP for the cyclic AMP cell surface receptor. Other evidence against direct competition with the cyclic AMP receptor is that the number of adenosine binding sites is about 50-fold greater than the number of cyclic AMP binding sites, and the observation that the effect of adenosine derivatives on cyclic AMP binding is noncompetitive at high cyclic AMP con-

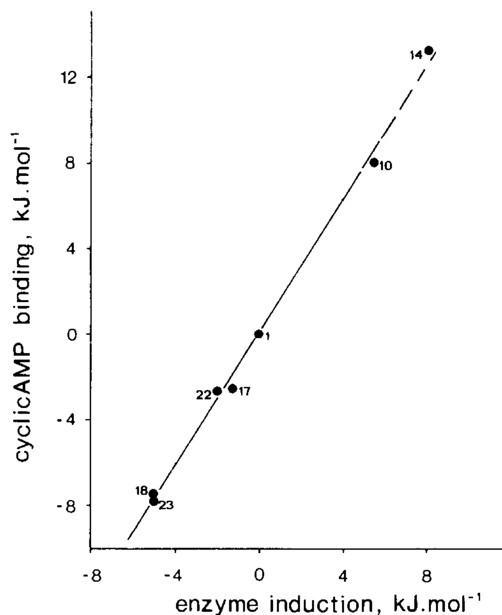

FIG. 5. Correlation between inhibition of cyclic AMP binding and inhibition of ornithine decarboxylase induction by several adenosine derivatives. Linear regression analysis yields a straight line with slope of 1.53 and correlation coefficient: $r = 0.996$, $P = 0.01\%, n = 6$. Data are taken from Table 1 and calculated from Fig. 4.

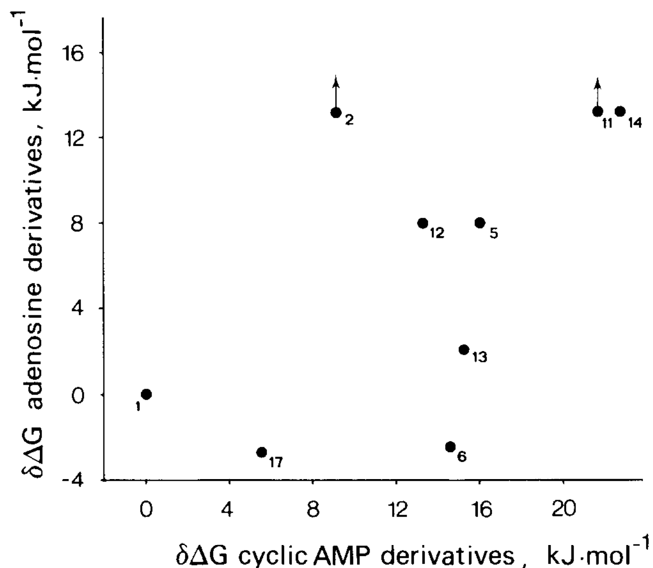

FIG. 6. Comparison of the effects of adenosine derivatives on the inhibition of cyclic AMP binding by adenosine with the effects of similarly modified cyclic AMP derivatives on cyclic AMP binding. $\delta\Delta G$ values for the inhibition of cyclic AMP binding by adenosine derivatives were taken from Table 1. $\delta\Delta G$ values for similarly modified cyclic AMP derivatives were taken from Van Haastert and Kien (1983). The numbers next to the points in the graph denote the type of modification as represented in Table 1. The arrows indicate that adenosine derivatives 2 and 11 do not inhibit cyclic AMP binding. The low correlation coefficient ($r = 0.59$, $P = >15\%, n = 7$) indicates that inhibition of cyclic AMP binding by adenosine is probably not caused by direct antagonism at the cyclic AMP receptor.

centrations (Van Haastert, 1983). This last point of competitiveness can, however, be debated as Theibert and Devreotes (1984) and Schaap and Wang (1986) have not found noncompetitive effects of adenosine at high cyclic AMP concentrations.

Role of Adenosine Uptake

The adenosine P site of higher organisms is located intracellularly and the effects mediated by this site can be abolished when uptake is inhibited. (Haslam and Rosson, 1975). In order to see whether the effects of adenosine on differentiation induction and cyclic AMP binding in *D. discoideum* are mediated through an internal site or a cell surface site, we investigated whether the inhibition of adenosine uptake could abolish the effect of adenosine on these responses.

Adenosine uptake can be effectively inhibitied by inosine and the nucleoside uptake inhibitor *S-p*-nitrobenzyl-6-thioinosine (Young and Jarvis, 1983) (see Fig. 7A) both of which have no detectable effect on the binding of cyclic AMP (see open symbols in Fig. 7D). Inhibition of adenosine uptake does not prevent the effect of adenosine on either cyclic AMP binding (Figs. 7C and D) or the induction of ornithine decarboxylase (Fig. 7B). It therefore appears that adenosine acts at the cell surface site in *D. discoideum.* The metabolism of adenosine is not considered to be of major importance for its effects, since the putative products inosine and 5'AMP are inactive (Table 1).

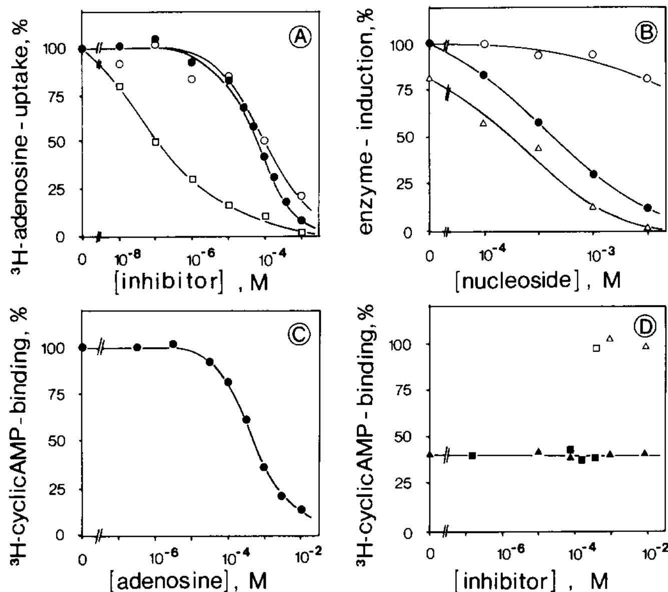

FIG. 7. Effect of adenosine uptake inhibitors on the action of adenosine on cyclic AMP binding and ornithine decarboxylase induction. (A) Inhibition of [^{3}H]adenosine uptake, measured as described under Materials and Methods, by different concentrations of inosine (○), adenosine (●), and *S-p*-nitrobenzyl-6-thioinosine (□). (B) Inhibition of ornithine decarboxylase induction, measured as described under Materials and Methods in the presence of 3×10^{-6} *M* (S_p)-cyclic AMPS, by different concentrations of inosine (○), adenosine (●), and adenosine together with 3×10^{-3} *M* inosine (△). (C) Dose-response curve of the effect of adenosine on [^{3}H]cyclic AMP binding, measured as described under Materials and Methods. (D) Effect of inosine (triangles) and *S-p*-nitrobenzyl-6-thioinosine (squares) on [^{3}H]cyclic AMP binding in the presence (filled symbols) or absence (open symbols) of 0.75 m*M* adenosine.

What Is the Function of Adenosine in D. discoideum?

In higher organisms, the P site is thought to be of limited importance due to its low affinity for adenosine, and the absence of sufficiently high adenosine concentrations. During aggregation of *D. discoideum* a similar situation seems to occur for the effects of adenosine that are mediated through the P-site-like receptor, because the extracellular adenosine concentration does not exceed the micromolar range in this stage (unpublished results).

After aggregation, sufficiently high concentrations of adenosine appear to accumulate for a physiological effect; this was demonstrated by treating slugs with adenosine deaminase to degrade endogenous adenosine (Schaap and Wang, 1986). This treatment induced the appearance of prespore-specific antigen in the entire prestalk region. This indicates that endogenous adenosine inhibits prespore specific differentiation in the prestalk region during normal development and has led to the proposal of a model in which adenosine is responsible for maintaining the integrity of the prestalk area by antagonizing the induction of prespore differentiation by cAMP (Schaap and Wang, 1986).

From the results presented in this paper and the results from Schaap and Wang (1986), it seems very likely that a physiologically functional receptor for adenosine, with P-site-like binding properties, is located at the surface of *D. discoideum* cells. The affinity of this receptor is very similar to that of the β receptor described previously (Newell, 1982; Van Haastert, 1983). An intriguing question which remains unsolved is the possible function of α adenosine receptor, which has an affinity in the micromolar range (Newell, 1982; Van Haastert, 1983). No effects of micromolar adenosine concentrations have yet been reported.

The close correlation between inhibition of cyclic AMP binding by adenosine and inhibition of cyclic-AMP-induced differentiation provides further evidence that the effect of cyclic AMP on differentiation induction is mediated through the cell-surface cyclic AMP receptor, and not through an intracellular cyclic AMP receptor.

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REFERENCES

ARMANT, D., STETLER, D., and RUTHERFORD, C. L. (1980). Cell surface localization of 5'-AMP nucleotidase in prestalk cells of *Dictyostelium discoideum*. *J. Cell Sci.* **45**, 119–129.

BARANIAK, J., KINAS, R. W., LESIAK, K., and STEC, W. J. (1979). Stereospecific synthesis of adenosine 3',5'-(S_p)- and (R_p)-cyclic phosphorothioates (cAMPS). *J. Chem. Soc. Chem. Commun.* 940–942.

BRENNER, M., and THOMS, S. D. (1984). Caffeine blocks activation of cyclic AMP synthesis in *Dictyostelium discoideum*. *Dev. Biol.* **101**, 136–146.

BROWN, S. S., and RUTHERFORD, C. L. (1980). Localization of cyclic nucleotide phosphodiesterase in the multicellular stages of *Dictyostelium discoideum*. *Differentiation* **16**, 173–184.

BRUNS, R. F. (1980). Adenosine receptor activation in human fibroblasts: Nucleoside agonists and antagonists. *Canad. J. Physiol. Pharmacol.* **58**, 673–691.

CHISHOLM, R. L., BARKLIS, E., and LODISH, H. F. (1984). Mechanism of sequential induction of cell-type specific mRNA in *Dictyostelium* differentiation. *Nature (London)* **310**, 67–69.

DALY, J. W. (1982). Adenosine receptors: Targets for future drugs. *J. Med. Chem.* **25**, 197–207.

DALY, J. W. (1985). Adenosine receptors. *Adv. Cyclic Nucl. Protein Phosphor. Res.* **19**, 29–46.

HASLAM, R. J., and ROSSON, G. M. (1975). Effects of adenosine on levels of adenosine cyclic 3',5'-monophosphate in human blood platelets in relation to adenosine incorporation and platelet aggregation. *Mol. Pharmacol.* **11**, 528–544.

JASTORFF, B., HOPPE, J., and MORR, M. (1979). A model for the chemical interactions of cAMP with the R-subunit of protein kinase type I. *Eur. J. Biochem.* **101**, 555–561.

KONIJN, T. M. (1970). Microbiological assay of cyclic 3',5'-AMP. *Experientia* **26**, 367–369.

LONDOS, C., and WOLFF, J. (1977). Two distinct adenosine sensitive sites on adenylate cyclase. *Proc. Natl. Acad. Sci. USA* **74**, 5482–5486.

LONDOS, C., COOPER, D. M. F., and WOLFF, J. (1980). Subclasses of external adenosine receptors. *Proc. Natl. Acad. Sci. USA* **770**, 2551–2554.

LOOMIS, W. F. (1975). *Dictyostelium discoideum. A developmental system.* Academic Press, New York.

LOOMIS, W. F., ed. (1982). *The Development of Dictyostelium discoideum.* Academic Press, New York.

NEWELL, P. C. (1982). Cell surface binding of adenosine to *Dictyostelium* and inhibition of pulsatile signalling. *FEMS Microbiol. Lett.* **13**, 417–421.

NEWELL, P. C., and ROSS, F. M. (1982). Inhibition by adenosine of aggregation centre initiation and cyclic AMP binding in *Dictyostelium*. *J. Gen. Microbiol.* **128**, 2715–2724.

PREMONT, J., PEREZ, M., BLANC, G., TASSIN, J.-P., THIERRY, A.-M., HERVE, D., and BLOCKAERT, J. (1979). Adenosine sensitive adenylase cyclase in rat brain homogenates: Kinetic characteristics, specificity, topographical, subcellular and cellular distribution. *Mol. Pharmacol.* **16**, 790–804.

SCHAAP, P., and SPEK, W. (1984). Cyclic AMP binding to the cell surface during development of *Dictyostelium discoideum*. *Differentiation* **27**, 83–87.

SCHAAP, P., and VAN DRIEL, R. (1985). The induction of post-aggregative development by cAMP in *Dictyostelium discoideum*. Evidence for the involvement of the cell surface receptor. *Exp. Cell Res.* **159**, 388–398.

SCHAAP, P., and WANG, M. (1986). Interactions between adenosine and oscillatory cAMP signalling regulate morphogenesis and pattern formation in *Dictyostelium*. *Cell*, in press.

THEIBERT, A., and DEVREOTES, P. N. (1984). Adenosine and its derivatives inhibit the cAMP signalling response in *Dictyostelium discoideum*. *Dev. Biol.* **106**, 166–173.

TOWN, C., and GROSS, J. (1978). The role of cyclic nucleotides and cell agglomeration in post aggregative enzyme synthesis in *Dictyostelium discoideum*. *Dev. Biol.* **63**, 412–420.

TSANG, A. S., and BRADBURY, J. M. (1981). Separation and properties of prestalk and prespore cells of *Dictyostelium discoideum*. *Exp. Cell Res.* **132**, 433–441.

VAN HAASTERT, P. J. M. (1983). Binding of cAMP and adenosine derivatives to *Dictyostelium discoideum* cells: Relationship between binding, chemotactic, and antagonistic activities. *J. Biol. Chem.* **258**, 9643–9648.

VAN HAASTERT, P. J. M., and KIEN, E. (1983). Binding of cAMP derivatives to *Dictyostelium discoideum* cells: Activation mechanism of the cell surface cAMP receptor. *J. Biol. Chem.* **258**, 9636–9642.

VAN LOOKEREN CAMPAGNE, M. M., and LÖWIK, C. G. W. M. (1985). The developmental regulation of ornithine decarboxylase in *Dictyostelium discoideum* and its induction by cAMP. *Biochim. Biophys. Acta* **846**, 55–63.

WEIJER, C. J., and DURSTON, A. J. (1985). Influence of cyclic AMP and hydrolysis products on cell type regulation in *Dictyostelium discoideum*. *J. Embryol. Exp. Morphol.* **86**, 19–37.

WOLFF, J., LONDOS, C., and COOPER, D. M. F. (1981). Adenosine receptors and the regulation of adenylate cyclase. *Adv. Cyclic Nucleotide Res.* **14**, 199–214.

YOUNG, J. D., and JARVIS, S. M. (1983). Nucleoside transport in animal cells. *Biosci. Rep.* **3**, 309–322.