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Competitive cAMP Antagonists for cAMP-Receptor Proteins*

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The two exocyclic oxygen atoms at phosphorus of cAMP have been replaced by a sulfur atom or by a dimethylamino group. These substitutions introduce chirality at the phosphorus atom; therefore, two diastereoisomers are known for each derivative: (S_P)-cAMPS, (R_P)-cAMPS, (S_P)-cAMPN(CH_3)$_2$, and R_P-cAMPN(CH_3)$_2$. We have investigated the agonistic and antagonistic activities of these compounds in four cAMP-dependent reactions: activation of the cellular slime mold *Dictyostelium discoideum* via its cell surface cAMP receptor, and phosphorylation by cAMP-dependent protein kinases type I, type II (both mammalian enzymes), and type D (derived from *D. discoideum*). The results show that 1) the compounds (S_P)-cAMPs and (S_P)-cAMPN(CH_3)$_2$ are (mostly full) agonists for the four proteins. Half-maximal activation is at micromolar concentrations (0.8–7 μM). 2) (R_P)-cAMPS is a full antagonist for the cell surface receptor and protein kinases type I and II, with apparent inhibition constants between 0.8 and 8 μM. This compound is a partial agonist for protein kinase type D, where it induces maximally 50% activation of the enzyme if compared with cAMP. 3) (R_P)-cAMPN(CH_3)$_2$ is a full antagonist for the cell surface receptor, and for protein kinase type II. This compound is a partial agonist for protein kinase type I (at least 50% activation if compared with cAMP), and inactive for protein kinase type D. This derivative is at least 25-fold less active as an antagonist than (R_P)-cAMPS. 4) The activity of mixtures of different concentrations of the antagonist (R_P)-cAMPS with different concentrations of cAMP reveals that the compound is a competitive antagonist of cAMP at micromolar concentrations.

cAMP has an important function as a signal molecule in hormone action and cell communication. During the last 20 years more than 600 derivatives of cAMP have been synthesized, with the aim to find compounds with special activities, such as nonhydrolyzable agonists or antagonists. The high number of derivatives prevents the screening of all compounds in many cAMP-dependent reactions. Therefore, we have used a different approach (1–3) in which it is assumed that cAMP interacts with its receptor proteins via electrostatic forces, such as ionic interactions, hydrogen bonds, and hydrophobic interactions. cAMP can form all types of interactions. A set of derivatives has been selected, in which each derivative prevents only one of the possible interactions while the other interactions are minimally disturbed. This method allows to obtain information on the major interactions between cAMP and its receptor proteins by using only 20 derivatives (4, 5).

Two types of experiments are performed in the most extensive studies. 1) The inhibition of the binding of [^{3}H]cAMP to the receptor proteins by the derivatives provides information on the atomic interactions between the cAMP molecule and the receptor protein. 2) The activity of the receptor-effector complex at saturating derivative concentrations provides information on the intrinsic activity of the derivative; *i.e.* the extent to which a derivative can activate when all the receptor sites are occupied with derivative. These studies indicate that essentially all derivatives of cAMP with modifications in the adenine, ribose, or cyclophosphate moiety fully activate the receptor (4, 5). Recently four new derivatives have been synthesized which show anomalous binding and activation profiles (4–6). In these derivatives the two exocyclic oxygen atoms of the cyclophosphate moiety have been replaced by a sulfur atom (7) or a dimethylamino group. These substitutions introduce chirality at the phosphorus atom of cAMP yielding two stereoisomers for each substitution: (S_P)-cAMPS,[1] (R_P)-cAMPS, (S_P)-cAMPN(CH_3)$_2$, and (R_P)-cAMPN(CH_3)$_2$ (see Fig. 1).

De Wit *et al.* (4) were first to report on the differential activation of cAMP-dependent protein kinase type I from rabbit muscle by (S_P)-cAMPS and (R_P)-cAMPS. They showed that binding of the S_P isomer to the enzyme yields complete activation of the protein kinase. In contrast, the R_P isomer could not activate the enzyme by more than 10%, although it occupied the enzyme by more than 90%. O'Brian *et al.* (6) subsequently reported similar data for cAMP-dependent protein kinase type II from rabbit muscle.

Recently we have used these compounds to reveal the activation mechanism of the cell surface cAMP receptor from the cellular slime mold *Dictyostelium discoideum* (5), and the mechanism of enzymatic hydrolysis of cAMP by phosphodiesterase (8). During these studies we observed that the preparations of the R_P isomers of cAMPS and cAMPN(CH_3)$_2$ contained traces of their S_P isomers (2–6%) and cAMP, which might be responsible for the activities of the R_P preparations reported previously (4, 6, 9). After extensive purification we

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[1] The abbreviations used are: cAMPS, adenosine 3′,5′-monophosphorothioate; cAMPN(CH_3)$_2$, adenosine 3′,5′-monophosphodimethylamidate; MES, 4-morpholineethanesulfonic acid.

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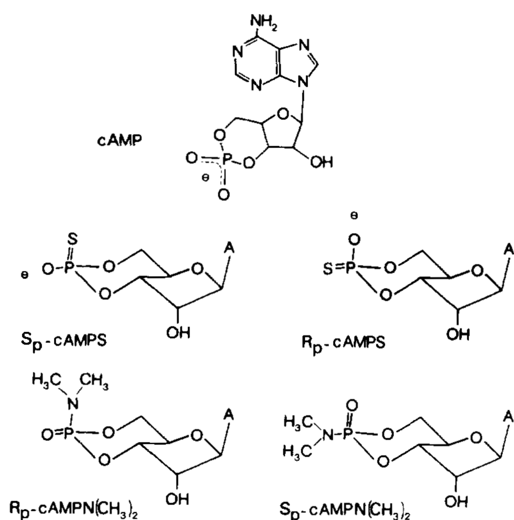

FIG. 1. **Structures of the cAMP analogs with modified exocyclic oxygen atoms.**

observed that the R_P isomers of cAMPS and cAMPN(CH_3)$_2$ no longer have activating properties. Since these compounds bind to the cAMP receptor in a competitive manner with respect to cAMP it was suggested that these compounds are antagonists of cAMP.

In the present work we have investigated the agonistic and antagonistic activities of the highly purified preparations of the S_P and R_P isomers of cAMPS and cAMPN(CH_3)$_2$ for four cAMP-dependent proteins: the cell surface cAMP receptor from *D. discoideum*, cAMP-dependent protein kinase type I from beef heart, type II from rabbit muscle, and type D from *D. discoideum*. The results show that antagonists are present among these four derivatives.

EXPERIMENTAL PROCEDURES

Materials—[γ-^{32}P]ATP was obtained from New England Nuclear and the radioimmunoassay kit from the Radiochemical Centre (Buckinghamshire, United Kingdom). Sephacryl S-300 was purchased from Pharmacia (Uppsala, Sweden); Leu-Arg-Arg-Ala-Ser-Leu-Gly (kemptide) was from Sigma.

The stereoisomers of cAMPS were synthesized as described in Ref. 7. The synthesis of the isomers of cAMPN(CH_3)$_2$ will be described elsewhere. The compounds were purified by high performance liquid chromatography (5). After purification, the R_P isomers are free of detectable levels of the S_P isomers or cAMP (< 0.1%).

Methods—The cAMP-mediated cGMP response was measured in aggregative *D. discoideum*, NC-4(H), cells (10). Briefly, 50-μl cell suspensions were stimulated with 10 μl of cAMP or derivative. The reaction was terminated after 10 s by the addition of 50 μl of perchloric acid. cGMP was measured in the neutralized lysates by means of a radioimmunoassay.

cAMP-dependent protein kinase type D (holoenzyme) was isolated from aggregative *D. discoideum*, AX-2, by gel filtration of a cytosolic cell fraction on Sephacryl S-300 as described in Ref. 11.

cAMP-dependent protein kinase type I from beef heart was isolated as described in Ref. 12 using one DEAE-chromatography step. cAMP-dependent protein kinase type II from rabbit muscle was obtained from Sigma. Protein kinase activity was measured (13) in a reaction mixture (60 μl) containing 50 mM MES buffer (pH 6.5), 5 mM $MgCl_2$, 0.5 mM EGTA, 10 mM NaF, 2.5 mM dithiothreitol, 20 μM kemptide, 0.2 mM [γ-^{32}P]ATP (2 Bq/pmol), enzyme, and cyclic nucleotides. The reaction (30 °C) was started by the addition of 30 μl of enzyme, and terminated after 10 min by the addition of 600 μl of a 50% slurry of Dowex 1-X2 in 30% acetic acid. After equilibration for 1 h, samples were centrifuged for 2 min at 10,000 × *g*. The radioactivity in 175 μl of the supernatant was determined.

RESULTS

The Cell Surface cAMP Receptor from D. discoideum—Binding of cAMP to the cell surface cAMP receptor of the cellular slime mold *D. discoideum* induces several responses, of which an intracellular accumulation of cGMP is the first response observed (for review on signal transduction in *D. discoideum* see Refs. 14 and 15). cGMP levels reach a peak at 10 s after stimulation and prestimulated levels are recovered within about 30 s. The pace of the cGMP response is the same for different cAMP analogs (10).

The cGMP response induced by cAMP and the four derivatives with modified exocyclic oxygen atoms are shown in Fig. 2*A*; three compounds are active, and two are inactive. Linear curves arise when these data are replotted according to Eadie and Hofstee (Fig. 2, *C* and *D*). The intersections with the ordinate reveal that cAMP, (S_P)-cAMPS, and (S_P)-cAMPN(CH_3)$_2$ induce about the same maximal response. The slopes of these curves represent the K_a (concentration which induces half-maximal stimulation); this yields 14 nM for

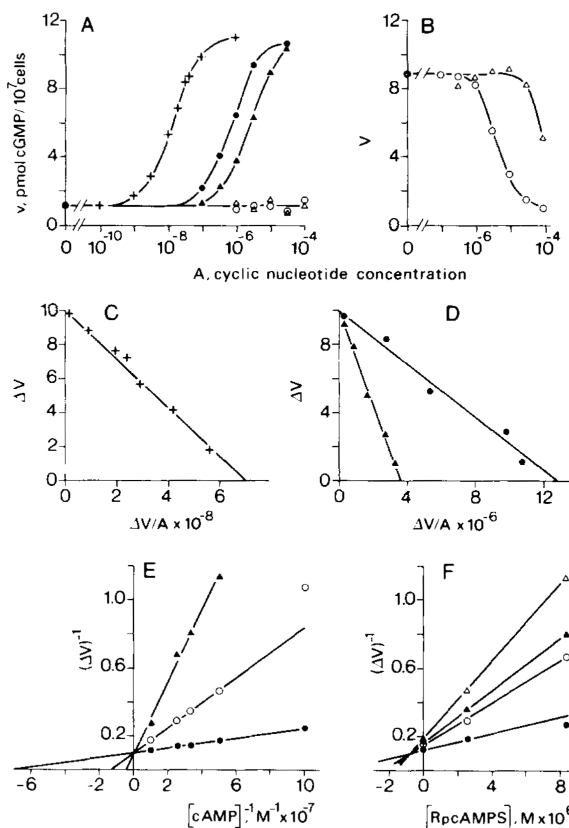

FIG. 2. **Agonistic and antagonistic activities of exocyclic oxygen-modified cAMP analogs for cAMP-mediated cGMP response in *D. discoideum*.** *A*, *D. discoideum* cells were stimulated with different concentrations of cAMP (+), (S_P)-cAMPS (●), (R_P)-cAMPS (○), (S_P)-cAMPN(CH_3)$_2$ (▲), or (R_P)-cAMPN(CH_3)$_2$ (△). Cells were lysed at 10 s after stimulation and cGMP levels were measured radioimmunologically. *B*. *D. discoideum* cells were preincubated for 30 s with different concentrations of (R_P)-cAMPS (○), (R_P)-cAMPN(CH_3)$_2$ (△). Then 50 nM cAMP was added, cells were lysed 10 s later, and cGMP levels were measured. *C* and *D*, Eadie-Hofstee plots of the data of *A*. ΔV is the increase of cGMP levels over basal levels, and *A* is the concentration of the nucleotides. The intersections with the *ordinates* are the maximal response; the slopes equal $-K_a$. Symbols are described in *A*. *E* and *F*, inspection for competitive antagonism of (R_P)-cAMPS. *D. discoideum* cells were preincubated for 30 s with three concentrations of (R_P)-cAMPS (0, 2.5, 8.33 μM). Then cells were stimulated with different cAMP concentrations (0, 10, 20, 30, 40, and 100 nM); cells were lysed 10 s later and cGMP levels were measured. A Lineweaver-Burk plot of the data is shown in *E*; preincubation without (●), or with 2.5 μM (○), or 8.33 μM (▲) (R_P)-cAMPS. A Dixon plot of the same data is shown in *F*; response to 100 nM (●), 40 nM (○), 30 nM (▲), or 20 nM (△) cAMP.

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cAMP, 0.78 μM for (S_P)-cAMPS, and 2.8 μM for (S_P)-cAMPN$(CH_3)_2$.

The compounds (R_P)-cAMPS and (R_P)-cAMPN$(CH_3)_2$ do not induce a cGMP response, although it has been shown that they bind to the cell surface cAMP receptors at micromolar concentrations (5). To test the compounds for antagonistic properties, the cells were mixed with the R_P stereoisomers and then stimulated with 50 nM cAMP. This reveals (Fig. 2*B*) that the analogs antagonize the stimulating activity of cAMP. Half-maximal inhibition (IC_{50}) occurs at 4 μM (R_P)-cAMPS and at about 100 μM (R_P)-cAMPN$(CH_3)_2$.

Since these compounds are investigated with whole cells they may inhibit the cAMP-mediated cGMP response at a site distinct from the cAMP receptor. Therefore, it had to be established that the R_P compounds are competitive antagonists of cAMP. Cells were stimulated by different cAMP concentrations in the presence of different concentrations of (R_P)-cAMPS. The Lineweaver-Burk plot (Fig. 2*E*) and the Dixon plot (Fig. 2*F*) indicate that (R_P)-cAMPS is a competitive antagonist of cAMP. The K_I values for the R_P isomers can be calculated from the IC_{50} values obtained in Fig. 2*B* by using the equation

$$K_I = IC_{50}\frac{K_a}{A + K_a}$$

where K_a is the activation constant of cAMP (14 nM) and A is the concentration of cAMP (50 nM). This yields $K_I = 0.9$ μM for (R_P)-cAMPS and $K_I \approx 22$ μM for (R_P)-cAMPN$(CH_3)_2$. K_I values can also be derived from a Dixon plot (Fig. 2*F*) which yields $K_I = 0.8$ μM for (R_P)-cAMPS.

These data demonstrate (Table I) that (S_P)-cAMPS and (S_P)-cAMPN$(CH_3)_2$ are full agonists of cAMP for the induction of a cGMP response in *D. discoideum*. (R_P)-cAMPS and (R_P)-cAMPN$(CH_3)_2$ are competitive full antagonists of cAMP. The effects of the derivatives with the cAMP-dependent protein kinases type I, II, and D were investigated in a similar manner.

cAMP-dependent Protein Kinases Type I and Type II from Mammalian Cells—The transfer of ^{32}P from [γ-^{32}P]ATP to kemptide catalyzed by protein kinase type II from rabbit muscle in the presence of cAMP and/or the derivatives is shown in Fig. 3. cAMP, (S_P)-cAMPS, and (S_P)-cAMPN$(CH_3)_2$ stimulate protein kinase type II, while both R_P isomers are inactive. An Eadie-Hofstee plot of these data (Fig. 3, *C* and *D*) yields a nonlinear concave upwards curve, which may suggest negative cooperativity. The analog (S_P)-cAMP-N$(CH_3)_2$ induces about the same maximal response as cAMP (intersection with an ordinate); the maximal activation induced by (S_P)-cAMPS is significantly less (activation up to 85%). This has also been shown previously by O'Brian *et al.* (6). The apparent activation constants (concentrations which induce half-maximal stimulation of protein kinase activity) are about 0.4 μM for cAMP, 1.8 μM for (S_P)-cAMPS, and 7.0 μM for (S_P)-cAMPN$(CH_3)_2$.

The two analogs, (R_P)-cAMPS and (R_P)-cAMPN$(CH_3)_2$, which do not stimulate protein kinase type II were tested for antagonistic activities; the enzyme was incubated with 2 μM cAMP and different concentrations of the R_P isomers (Fig. 3*B*). The stimulating effect of cAMP is antagonized by (R_P)-cAMPS with an IC_{50} of about 16 μM. The analog (R_P)-cAMPN$(CH_3)_2$ has much less antagonizing activity with an estimated IC_{50} of ≈ 450 μM.

To test whether these compounds are competitive antagonists of cAMP is complicated by the fact that activation of protein kinase type II does not follow Michaelis-Menten kinetics; this leads to nonlinear Eadie-Hofstee plots (Fig. 3, *C* and *D*) and now linear Lineweaver-Burk and Dixon plots (data not shown). The activity of the enzyme in the presence of different concentrations of cAMP and (R_P)-cAMPS are shown in Fig. 3, *E* and *F*. The dose-response curves run parallel, which is the first indication for competitive antagonism. In a Hill plot of these data (Fig. 3*F*) it was assumed that the enzyme has the same ΔV_{max} in the absence and presence of the antagonist (this is a prerequisite for compet-

TABLE I

Properties of cAMP and cAMP derivatives for four cAMP receptor proteins

	CSR D[a]	CAK I[b]	CAK II[b]	CAK D[b]
Affinity, $K_{0.5}$ (μM)				
cAMP	0.014	0.028	0.4	0.15
(S_P)-cAMPS	0.78	1.0	1.78	1.9
(R_P)-cAMPS	Inactive	Inactive	Inactive	11.3
(S_P)-cAMPN$(CH_3)_2$	2.78	2.24	7.0	3.2
(R_P)-cAMPN$(CH_3)_2$	Inactive	≈1000	Inactive	Inactive
Hill coefficient	1.0	1.8	0.8	1.0
Maximal response (cAMP = 100)				
(S_P)-cAMPS	100	100	85	100
(R_P)-cAMPS	<5	<5	<5	52
(S_P)-cAMPN$(CH_3)_2$	100	76	100	100
(R_P)-cAMPN$(CH_3)_2$	<5	>50	<5	<5
Antagonism K_I (μM)				
(R_P)-cAMPS	0.8	8.0	3.7	Agonist
(R_P)-cAMPN$(CH_3)_2$	22	Agonist	≈75	Inactive
Classification				
(S_P)-cAMPS	Agonist	Agonist	Part. agonist	Agonist
(R_P)-cAMPS	Antagonist	Antagonist	Antagonist	Part. agonist
(S_P)-cAMPN$(CH_3)_2$	Agonist	Part. agonist	Agonist	Agonist
(R_P)-cAMPN$(CH_3)_2$	Antagonist	Agonist	Antagonist	Inactive

[a] Cell surface cAMP receptor for *D. discoideum*.
[b] cAMP-dependent protein kinase.

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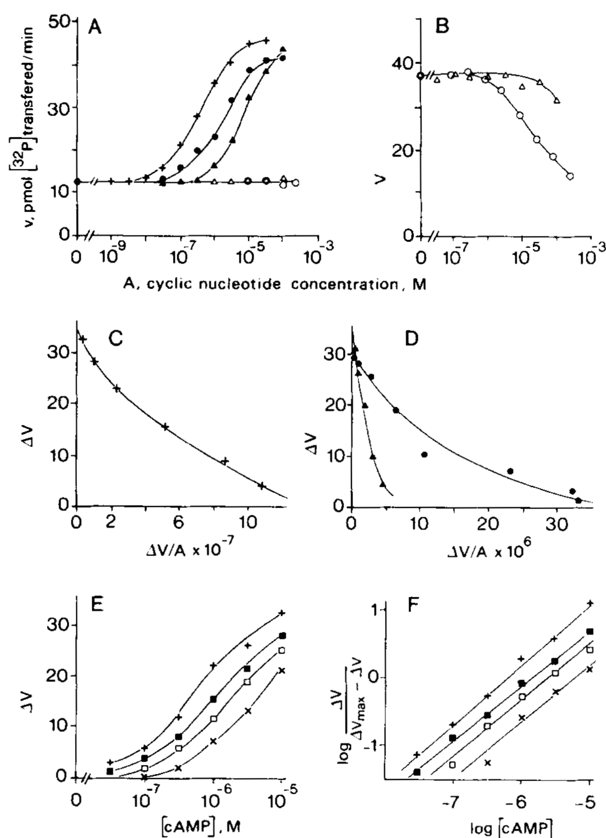

FIG. 3. **Agonistic and antagonistic activities of exocyclic oxygen-modified cAMP analogs for cAMP-dependent protein kinase type II.** *A*, the transfer of ^{32}P from [γ-^{32}P]ATP to kemptide catalyzed by cAMP-dependent protein kinase type II from rabbit muscle was measured in the presence of different concentrations of cAMP (+), (S_P)-cAMPS (●), (R_P)-cAMPS (○), (S_P)-cAMPN(CH_3)$_2$ (▲), or (R_P)-cAMPN(CH_3)$_2$ (△). *B*, protein kinase activity in the presence of 2 μM cAMP and different concentrations of (R_P)-cAMPS (○), or (R_P)-cAMPN(CH_3)$_2$ (△). *C* and *D*, Eadie-Hofstee plots of the data of *A*. In ΔV the protein kinase activity in the absence of cyclic nucleotide is subtracted. Symbols are as described in *A*. *E* and *F*, inspection for competitive antagonism of (R_P)-cAMPS. Activation of protein kinase type II by different cAMP concentrations was measured in the absence (+), or in the presence of 2.5 μM (■), 14 μM (□), or 50 μM (×) (R_P)-cAMPS. Dose-response curves are shown in *E*. A Hill plot from the same data is shown in *F*; ΔV_{max} is the maximal increase of activity induced by cAMP (intersection with ordinate in *C*).

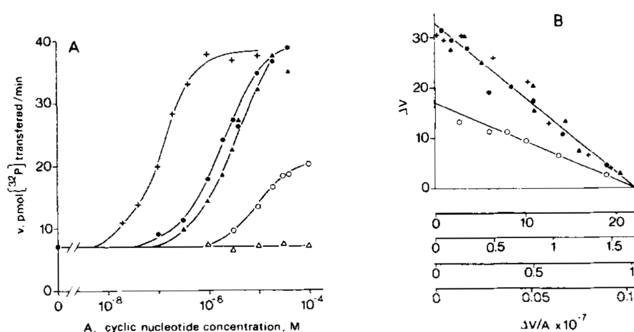

FIG. 4. **Agonistic activities of exocyclic oxygen-modified cAMP derivatives for cAMP-dependent protein kinase type D.** *A*, partially purified protein kinase was isolated from *D. discoideum* cells. Protein kinase activity was measured in the presence of different concentrations of cAMP (+), (S_P)-cAMPS (●), (R_P)-cAMPS (○), (S_P)-cAMPN(CH_3)$_2$ (▲), or (R_P)-cAMPN(CH_3)$_2$ (△). *B*, Eadie-Hofstee plot of the same data. The *abscissas* are scaled in such a way that the four curves converge into a single point. The fact that +, ●, and ▲ are part of the same curve indicates that these compounds induce the same maximal response. Symbols are as described in *A*.

itive antagonism). The four lines in the Hill plot have essentially identical slopes (Hill coefficient, $n = 0.8$). This strongly suggests that the compound (R_P)-cAMPS is a competitive antagonist of cAMP for protein kinase type II. The data indicate an apparent $K_I = 3.7$ μM for (R_P)-cAMPS and $K_I \approx$ 75 μM for (R_P)-cAMPN(CH_3)$_2$. The results with cAMP-dependent protein kinase type II indicate (Table I) that (S_P)-cAMPN(CH_3)$_2$ is a full agonist of cAMP, (S_P)-cAMPS is a partial agonist, (R_P)-cAMPN(CH_3)$_2$ is an antagonist, and (R_P)-cAMPS is a competitive full antagonist.

The same experiments were done with cAMP-dependent protein kinase type I from beef heart, which yields qualitatively similar, but quantitatively different results (data not shown). The compounds cAMP, (S_P)-cAMPS, and (S_P)-cAMPN(CH_3)$_2$ activate the enzyme with apparent activation constants $K_{0.5}$ = 28 nM for cAMP, $K_{0.5}$ = 1.0 μM for (S_P)-cAMPS, and $K_{0.5}$ = 2.2 μM for (S_P)-cAMPN(CH_3)$_2$. In contrast to protein kinase type II, the compound (R_P)-cAMPN(CH_3)$_2$ is also stimulatory in protein kinase type I with an estimated $K_{0.5} \approx$ 1 mM. Eadie-Hofstee plots are nonlinear (concave downwards), which suggests positive cooperativity. This is also suggested by the Hill coefficient $n = 1.8$. cAMP and (S_P)-cAMPS induces about the same maximal activity; the maximal activity induced by (S_P)-cAMPN(CH_3)$_2$ is significantly less (75% of the maximum of cAMP). The analog (R_P)-cAMPS antagonizes the stimulatory effect of 50 nM cAMP with an IC_{50} = 18 μM. Experiments with different concentrations of cAMP and (R_P)-cAMPS (analogous to the experiment shown in Fig. 3, *E* and *F*) indicate that (R_P)-cAMPS is a competitive antagonist of cAMP with an apparent K_I = 8 μM. The results show (Table I) that (S_P)-cAMPS is a full agonist of cAMP for protein kinase type I, (S_P)-cAMPN(CH_3)$_2$ is a partial agonist, and (R_P)-cAMPS is a competitive full antagonist. The compound (R_P)-cAMPN(CH_3)$_2$ is at least a partial agonist, since half-maximal stimulation is observed at 1 mM (the highest concentration investigated).

cAMP-dependent Protein Kinase from D. discoideum—Recently a cAMP-dependent protein kinase has been isolated from the cellular slime mold *D. discoideum* (type D) (11, 16, 17). cAMP, (S_P)-cAMPS, and (S_P)-cAMPN(CH_3)$_2$ activate this enzyme (Fig. 4*A*). Eadie-Hofstee plots show that these compounds induce approximately the same maximal activation of the enzyme (Fig. 4*B*). The compound (R_P)-cAMPS is also stimulatory, but its maximal activation is only 52% of the maximum induced by cAMP. Eadie-Hofstee plots are linear, which suggests the absence of cooperativity. The activation constants are 150 nM for cAMP, 1.9 μM for (S_P)-cAMPS, 11.3 μM for (R_P)-cAMPS, and 3.2 μM for (S_P)-cAMPN(CH_3)$_2$.

The compound (R_P)-cAMPN(CH_3)$_2$ does not activate the enzyme up to 100 μM (Fig. 4*A*), and it also does not inhibit the stimulation by 0.5 μM cAMP (data not shown). It has been shown previously (18) that this compound competes very poorly with the binding of [^3H]cAMP to the regulatory subunit of the enzyme. This suggests that (R_P)-cAMPN(CH_3)$_2$ is inactive.

The results show (Table I) that (S_P)-cAMPS and (S_P)-cAMPN(CH_3)$_2$ are full agonists of cAMP for protein kinase type D, (R_P)-cAMPS is a partial agonist, and (R_P)-cAMPN(CH_3)$_2$ is inactive.

DISCUSSION

In previous experiments on the interaction of cAMP derivatives with cAMP receptor proteins it was shown that essen-

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tially all derivatives that bind to the receptors also activate these receptors (4, 5). Studies on the action of the derivatives with modified exocyclic oxygen atoms revealed that these compounds bind to the receptors, but that some of them do not fully activate the receptors (4–6). This suggests that binding of cAMP to the receptor and activation of the receptor are two distinct processes in a concerted reaction. Binding of cAMP to a receptor involves atoms or atom groups distributed all over the cAMP structure, whereas activation requires an additional interaction between the receptor protein and the phosphate moiety of cAMP (4, 5). At least three atomic interactions are possible between the receptor and the phosphate moiety (i) a charge-charge interaction between a positively charged amino acid side chain and the negatively charged phosphate moiety (ii) a polar interaction (hydrogen bond) between one or both of the exocyclic oxygen atoms of cAMP and the receptor protein, and (iii) a covalent bond between the phosphorus atom and the receptor protein (19). A charge-charge interaction is excluded by the results with the noncharged analog (S_P)-cAMPN$(CH_3)_2$.

The proposed activation mechanisms led us to hypothesize that an analog with a modified exocyclic oxygen atom may still bind to the receptor, but is no longer able to provide the activating interaction. Such a derivative would be the much sought after antagonist of cAMP acting specifically at cAMP receptor proteins.

In this study we have investigated the antagonistic activities of four derivatives in which one of the exocyclic oxygen atoms is replaced by either a sulfur atom or a dimethylamino atom group, (S_P)-cAMPS, (R_P)-cAMPS, (S_P)-cAMPN$(CH_3)_2$ and (R_P)-cAMPN$(CH_3)_2$. These analogs were tested with four cAMP-dependent proteins: the cell surface receptor on intact *D. discoideum* cells which induces an intracellular cGMP response, cAMP-dependent protein kinase type I from beef heart, type II from rabbit muscle, and type D from *D. discoideum*.

The results show that (S_P)-cAMPS and (S_P)-cAMP-N$(CH_3)_2$ are (mostly full) agonists of cAMP. (R_P)-cAMPS does not activate cell surface receptors, protein kinase type I and type II. Previously a small activation (about 10%) of type I and type II was observed (4, 6), which now appears to be due to impurities of (S_P)-cAMPS (about 2%) and cAMP (less than 0.1%). These impurities (as well as impurities in (R_P)-cAMPN$(CH_3)_2$ were removed in the preparations used in the present study, and in previous reports on the cell surface cAMP receptor (5, 20).

In the present work we show that (R_P)-cAMPS is completely inactive and that it inhibits the stimulating effect of cAMP in a competitive way. The inhibition constants of (R_P)-cAMPS for the receptor proteins are similar to respective binding affinities of the analog for the receptors (4, 5, 8). Therefore, we conclude that (R_P)-cAMPS binds to the cAMP-binding sites, but that the analog is not able to activate the receptor; (R_P)-cAMPS is a competitive full antagonist of cAMP acting at cAMP-binding sites from the cell surface cAMP receptor, and protein kinase type I and type II. Interestingly, (R_P)-cAMPS partially activates protein kinase type D. This protein kinase from *D. discoideum* differs from mammalian protein kinases by molecular weight, subunit composition, and kinetic properties of cAMP-binding (11, 18). Nevertheless, the regulatory subunit can combine with the purified catalytic subunit from protein kinase type I or type II (21).

The analog (R_P)-cAMPN$(CH_3)_2$ is an antagonist for the cell surface cAMP receptor and for protein kinase type II. In contrast, the analog is an agonist for protein kinase type I, and inactive for protein kinase type D. We have not investigated the competitive nature of this antagonist, because the compound acts only at high concentrations.

Thus, we have shown that (R_P)-cAMPS is a competitive antagonist at micromolar concentration, acting specifically at cAMP-binding sites. However, the compound cannot be used without any precautions. First, (R_P)-cAMPS does not antagonize all cAMP-dependent proteins. cAMP-dependent protein kinase from *D. discoideum* is activated up to 50% by the analog, and the analog is the most potent activator of β-galactosidase synthesis in *Escherichia coli* (22). Second, the analog binds to mammalian phosphodiesterase, but is not hydrolyzed by the enzyme (8). Therefore, phosphodiesterase is partially inhibited, which allows endogenous cAMP to accumulate, and to compete with (R_P)-cAMPS for binding to protein kinase. Despite these complicating properties, recent reports on cellular slime mold chemotaxis (20) and on glycogenolysis (23) show the usefulness of the compound.

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