# THE CHEMOTACTIC ACTIVITY OF CYCLIC AMP AND AMP DERIVATIVES WITH SUBSTITUTIONS IN THE PHOSPHATE MOIETY IN *DICTYOSTELIUM DISCOIDEUM*

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## 1. Introduction

Cyclic AMP mediates cell aggregation in *Dictyostelium discoideum* [1]. During aggregation-competence differentiation, amoebae develop a cell-surface-bound cyclic AMP chemoreceptor [2–5]. The interaction of cyclic AMP with the chemoreceptor can be studied by triggering cells in their sensitive stage with an external source of cyclic AMP and monitoring the amoebae response [6]. By means of this technique it has been concluded that the cyclic AMP chemoreceptor in *D. discoideum* is very sensitive to structural changes in the cyclic AMP molecule specially at the phosphate moiety [7]. Also, it has been suggested that at the aggregative stage of this species only cyclic nucleotides show chemotactic acitivity [8]. In this paper further evidence is given on the essential role of the phosphate moiety in the interaction of cyclic AMP with the chemoreceptor. Surprisingly chemotactic activity is found with several AMP derivatives despite their non-cyclic structure. These results are discussed in the light of our present knowledge on chemotaxis in *D. discoideum.*

## 2. Materials and methods

Amoebae of *Dictyostelium discoideum* were cultured and the chemotactic assays were carried out as described previously [6]. Briefly: small droplets of an amoebae suspension were plated on a hydrophobic agar surface and the chemotactic activity of the various nucleotides tested after the amoebae reached their sensitive stage. Different nucleotide dilutions (0.1 $\mu$l) were deposited three-times at 5 min intervals at a distance of about 0.3 mm from the amoebae drops. Five min after the last deposition, the amoebal response was observed and considered positive when at least two-times as many amoebae were pressed against the side closer to the nucleotide as against the opposite side. The two dilutions of a nucleotide between which 50% of the amoebae drops reacted positively was taken as the threshold concentration. Adenosine 3′,5′-monophosphorothioate (cAMPS), adenosine 5′-methylmonophosphate (5′-AMPMe) and adenosine 5′-thiomethylmonophosphate (5′-AMPSMe) were kindly provided by Dr F. Eckstein. 3′-Deoxy-3′-aminoadenosine 3′,5′-monophosphate (3′-NH-cAMP), 5′-deoxy-5′-amino adenosine 3′-monophosphate (5′-$NH_2$-3′-AMP), 5′-deoxy-5′-aminomethyl adenosine 3′-monophosphate (5′-NHMe-3′-AMP) and 3′-deoxy-3′-amino adenosine 5′-monophosphate (3′-$NH_2$-5′-AMP) were kindly provided by Dr B. Jastorff. 5′-Deoxy-5′-thio adenosine 3′,5′-monophosphate (5′-*S*-cAMP) was kindly provided by Dr J. P. Miller (ICN Irvine, Calif.). Adenosine 3′,5′-monophosphate (cyclic AMP) was purchased from Boehringer and adenosine 5′-monophosphate (5′-AMP) and adenosine 3′-monophosphate (3′-AMP) were purchased from Sigma.

## 3. Results

### 3.1. *Chemotactic activity of the cyclic AMP derivatives*

The threshold activity of cAMPS was in the same range as cyclic AMP (fig.1). 3′-NH-cAMP was about

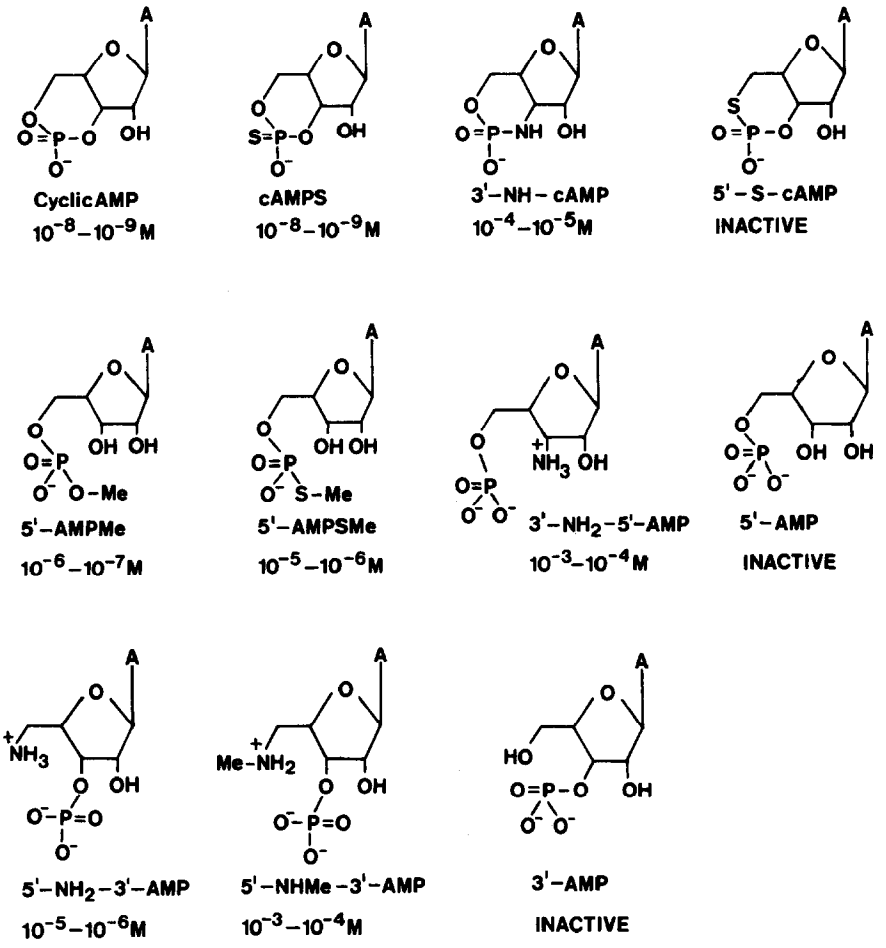


Fig.1. Chemotactic threshold activities of cyclic AMP and AMP derivatives with substitutions in the phosphate moiety.

$10^4$-times less active than cyclic AMP and 5′-*S*-cAMP was inactive up to a concentration of $10^{-4}$ M (fig.1).

### 3.2. *Chemotactic activity of the non-cyclic nucleotides*

While 5′-AMP and 3′-AMP were both inactive up to a concentration of $10^{-3}$ M (fig.1), the methyl ester derivatives of 5′-AMP was only about $10^2$-times less active than cyclic AMP (fig.1). Additional replacement of the oxygen involved in the methyl ester bond by sulphur, resulted in a reduction of the threshold activity by a factor of 10 (fig.1).

The substitution of the 3′-hydroxy group of the furanose moiety in the molecule of 5′-AMP by an amino group resulted in a chemotactically active molecule (3′-$NH_2$-5′-AMP) with a threshold activity from $10^{-3}$–$10^{-4}$ M (fig.1). The 3′-AMP derivative, 5′-$NH_2$-3′-AMP, was even more active than its isomer (3′-$NH_2$-5′-AMP) with a threshold activity from $10^{-5}$–$10^{-6}$ M (fig.1). The introduction of a methyl group to the amino group in the 5′-$NH_2$-3′-AMP molecule reduced the chemotactic activity by a factor of $10^2$ (fig.1).

## 4. Discussion

The phosphate moiety seems to be an essential part for the chemotactic activity of the cyclic AMP molecule. While the substitution of the 5′-oxygen in

the phosphate ring of the cyclic AMP molecule by a methylene group gives only a slight loss in chemotactic activity, the same substitution at the 3′-oxygen decreases its chemotactic activity by a factor of $10^6$ [8]. A similar preservation of activity has been observed by substitution of the 5′-oxygen by an amino group [7], while the same substitution at the 3′-oxygen decreased its activity by a factor of $10^4$ (fig.1). The lack of chemotactic activity observed with 5′-*S*-cAMP is most striking. This analogue is almost as active as cyclic AMP in protein kinase activation and as a substrate for phosphodiesterase hydrolysis [9]. These differences between the cyclic AMP chemoreceptor and other systems in relation to protein kinase and phoshphodiesterase activities have been already noticed with compounds as cyclic GMP and cyclic IMP, which are only 10–100-times less active than cyclic AMP in other systems and about $10^4$-times less active in chemotaxis [8]. From these results it seems that the cyclic AMP chemoreceptor in *D.discoideum* does not differentiate between an oxygen atom, a methylene or an amino group at the 5′-position of the phosphate ring, but is most sensitive to the introduction of a sulphur atom at this position. On the other hand, the 3′-position of the phosphate ring shows a much higher degree of specificity, which can be summarized according to the following order of activity:

$$0(3') >> NH(3') > CH_2(3')$$

Since the conformation of the phosphate ring is not dramatically changed by the replacement of an oxygen by a methylene or amino group [10,11] differences in activity within the various 3′-derivatives must be explained by a direct interaction between the cyclic AMP chemoreceptor and the 3′-oxygen. It is evident that to trigger chemotaxis the 3′-position of the phosphate ring is one of the most if not the most important site of the cyclic AMP molecule. A similar conclusion has been outlined from the work on protein kinase activation and on phosphodiesterase [11].

The substitution in the phosphate moiety of the double bonded oxygen by a sulphur atom preserved the chemotactic activity of the molecule of cyclic AMP. cAMPS is also comparable to cyclic AMP as protein kinase activator [12,13] and amylase secretion inductor [14], but is about 100-times less active than cyclic AMP as a phosphodiesterase substrate with either bovine [12,13] or *D.discoideum* [15] enzymes. As an agonist of the receptor, cAMPS is not more than 10-times less active than cyclic AMP [15]. Chemotactically cAMPS is about $10^2$-times more active than its related derivative 5′-deoxy-5′-amino adenosine 3′,5′-monophosphorothioate [7].

While 5′-AMP, 3′-AMP, adenine, ADP and ATP are inactive, it is surprising that various AMP derivatives are chemotactically active. The 5′-$NH_2$-3′-AMP was about $10^3$-times less active (fig.1) than its related cyclic nucleotide: 5′-NH-cAMP [7]. 5′-$NH_2$-3′-AMP is also active with protein kinase and may achieve a cyclic conformation by internal salt formation [11]. This cyclic conformation is not possible with 3′-$NH_2$-5′-AMP [11]. However, this AMP derivative was only 10-times less active than its related cyclic amido nucleotide (fig.1). These deoxy-amino-AMP derivatives do not inhibit cyclic AMP hydrolysis by phosphodiesterase [11]. The introduction of a methyl group in the amino group of the molecule of 5′-$NH_2$-3′-AMP decreased its chemotactic activity by a factor of $10^2$. This result agrees with previous observations which have shown that the cyclic AMP chemoreceptor is very sensitive to stereochemical modifications at the 5′-position of the phosphate ring [7]. The phosphorotioate mentioned in [16] was actually the AMP derivative 5′-AMPSMe although a less pure preparation than that used in the present paper [13].

Although 5′-AMPSMe is neither inhibitor nor substrate of brain and beef-heart phosphodiesterase [17] it showed chemotactic activity. The methyl ester of 5′-AMP was only $10^2$-times less active than cyclic AMP despite its non-cyclic structure. Due to the structural formulae of 5′-AMPSMe and 5′-AMPMe and to the results with brain and beef-heart phosphodiesterase [17] it seems justified to assume that these AMP derivatives may be resistant to the action of *D.discoideum* phosphodiesterase. Therefore, these results agree with those of Gerisch et al. [15] which indicate that the hydrolysis of a cyclic nucleotide is not prerequisite for its chemotactic action. So far it is not known whether these methyl AMP derivatives are also protein kinase activators. The chemotactic activity of the AMP derivatives was in the same range or stronger than the chemotactic activity of certain cyclic nucleotides as cyclic GMP or cyclic IMP [8].

A conformation analysis of the active non-cyclic nucleotides in comparison with their related cyclic and inactive non-cyclic nucleotides may be helpful for a better understanding of the nature of the cyclic AMP–chemoreceptor interaction. Finally, the present results with the non-cyclic nucleotides have shown that a cyclophosphate ring is not an essential part to trigger chemotaxis. In other words, that the chemoreceptor affinity can not be based on the opening of the cyclophosphate.

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## References

[1] Konijn, T. M., Barkley, D. S., Chang, Y. Y. and Bonner, J. T. (1968) Am. Nat. 102, 225–233.
[2] Malchow, D. and Gerisch, G. (1974) Proc. Natl. Acad. Sci. USA 71, 2423–2427.
[3] Mato, J. M. and Konijn, T. M. (1975) Biochim. Biophys. Acta 385, 173–179.
[4] Henderson, E. J. (1975) J. Biol. Chem. 250, 4730–4736.
[5] Green, A. A. and Newell, P. C. (1975) Cell 6, 129–136.
[6] Konijn, T. M. (1970) Experientia 26, 367–369.
[7] Konijn, T. M. and Jastorff, B. (1973) Biochim. Biophys. Acta 304, 774–780.
[8] Konijn, T. M. (1974) Antibiot. Chemother. 19, 96–110.
[9] Simon, L. N., Shuman, D. A. and Robins, R. K. (1973) Adv. Cyclic Nucleotide Res. 3, 225–353.
[10] Watenpaugh, K., Dow, J., Jensen, L. H. and Furberg, S. (1968) Science 159, 206–207.
[11] Panitz, N., Rieke, E., Morr, M., Wagner, K. G., Roesler, G. and Jastorff, B. (1975) Eur. J. Biochem. 55, 415–422.
[12] Eckstein, F., Simonson, L. P. and Bär, H. P. (1974) Biochemistry 13, 3806–3810.
[13] Eckstein, F. (1975) Angew. Chem. 14, 160–166.
[14] Eckstein, F., Eimerl, S. and Schramm, M. (1976) FEBS Lett. 64, 92–94.
[15] Gerisch, G. and Malchow, D. (1976) Adv. Cyclic Nucleotide Res. 7, 49–68.
[16] Konijn, T. M. (1972) Adv. Cyclic Nucleotide Res. 1, 17–31.
[17] Eckstein, F. and Bär, H. P. (1969) Biochim. Biophys. Acta 191, 316–321.