THE CHEMOTACTIC EFFECT OF CYCLIC NUCLEOTIDES WITH SUBSTITUTIONS IN THE BASE RING

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1. Introduction

The widespread role of cyclic AMP in the control of biochemical processes in the cell is well established. Other cyclic nucleotides and several analogues of cyclic AMP have been synthesized to study its complex effects. Substitutions have been introduced in the base, ribose, and phosphate moiety of cyclic AMP resulting in analogues whose activity is mostly less than that of cyclic AMP but sometimes exceeds its activity. Also analogues of the other cyclic nucleotides have been synthesized.

The amoebae of *Dictyostelium discoideum* are very suitable to test the activity of analogues of cyclic nucleotides. Cyclic AMP is the natural chemotactic agent responsible for cell aggregation in the larger species of *Dictyostelium* [1]. Free amoebae, close to the aggregative stage, are responsive to very low concentrations of cyclic AMP. Since the attractants probably activate the cell membrane (P.B. Moens, personal communication), and therefore act like hormones, the rate of penetration of the analogues into the cell does not obscure their actual effective concentrations.

Recently we studied the chemotactic effect of several 5′-amido analogues of cyclic AMP and found that the molecular receptor systems for cyclic AMP on the amoebae are highly sensitive to stereochemical alteration at the 5′-position of the cyclophosphate ring [2].

In this communication the chemotactic effect of cyclic nucleotides with alterations in the base moiety have been described.

2. Materials and methods

2.1. *Bio-essay*

Amoebae of *D. discoideum* were suspended in a salt solution and deposited as small drops on a hydrophobic agar surface [3]. All cells moved freely on the agar inside the margins of the drop and no amoebae crossed the boundaries of the drop.

The chemotactic activity of the various analogues was tested by depositing small droplets (0.1 μl) of the dissolved analogue at a distance of about 0.3 mm from populations of sensitive amoebae [3]. The various dilutions of the analogues were deposited three times at 5 min intervals. Five and 15 min after the last deposition, the response of the amoebae was observed through a phase contrast microscope at a magnification of 80×. A response was marked positive if at least two times as many amoebae were pressed against the side closest to the analogue as against the opposite side. The two dilutions of an analogue between which 50% of the amoebae populations reacted positively represented the threshold activity. The response of at least 20 amoebae populations to each of the various dilutions of an analogue was observed. The experiments were carried out in 4-fold or in 8-fold.

2.2. *Chemicals*

All components tested were kindly provided by Drs. Nelboeck and Michal of the Boehringer Co., Mannheim.

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3. Results and discussion

The chemotactic activities of N^6-benzoyl-cyclic AMP and 8-piperidino-cyclic AMP were highest among the tested analogues (fig. 1). Despite the change in stereochemistry and the electron density by the addition of a benzoyl or a piperidine ring to the adenine base the activity of these analogues was only an order of 10-fold less than the cyclic AMP activity. The threshold activity of N^6-benzyl-cyclic AMP was $10^{-6}-10^{-7}$ M.

A substitution of hydrogen at the 8-position of the adenine base by bromine reduced the chemotactic activity by a factor of 10^3 if compared with cyclic AMP (fig. 1). The threshold activity of tubercidin-cyclic phosphate [4], which molecule differs from cyclic AMP by the substitution of the nitrogen at the 7-position by carbon, also was $10^{-5}-10^{-6}$ M.

The activity of AICAR-cyclic MP was surprisingly high (fig. 1) if one considers its base which differs considerably from the adenine moiety of cyclic AMP. The activity of AICAR-cyclic MP exceeded that of cyclic CMP, cyclic GMP, cyclic IMP, cyclic TMP [4] and cyclic XMP.

Substitution of the hydrogen atom at the 8-position of the guanine base by bromine also produced a less active attractant than cyclic GMP itself (fig. 1).

This reduction in activity was small if compared with the 10^3-fold difference in activity between 8-bromo-cyclic AMP and cyclic AMP. We shoud consider, however, the fact that the activity of cyclic GMP is in the order of 10^3-fold lower than that of cyclic AMP.

Substitution of the hydrogen at the 8-position of cyclic GMP by a benzylamino group reduced the activity further (fig. 1).

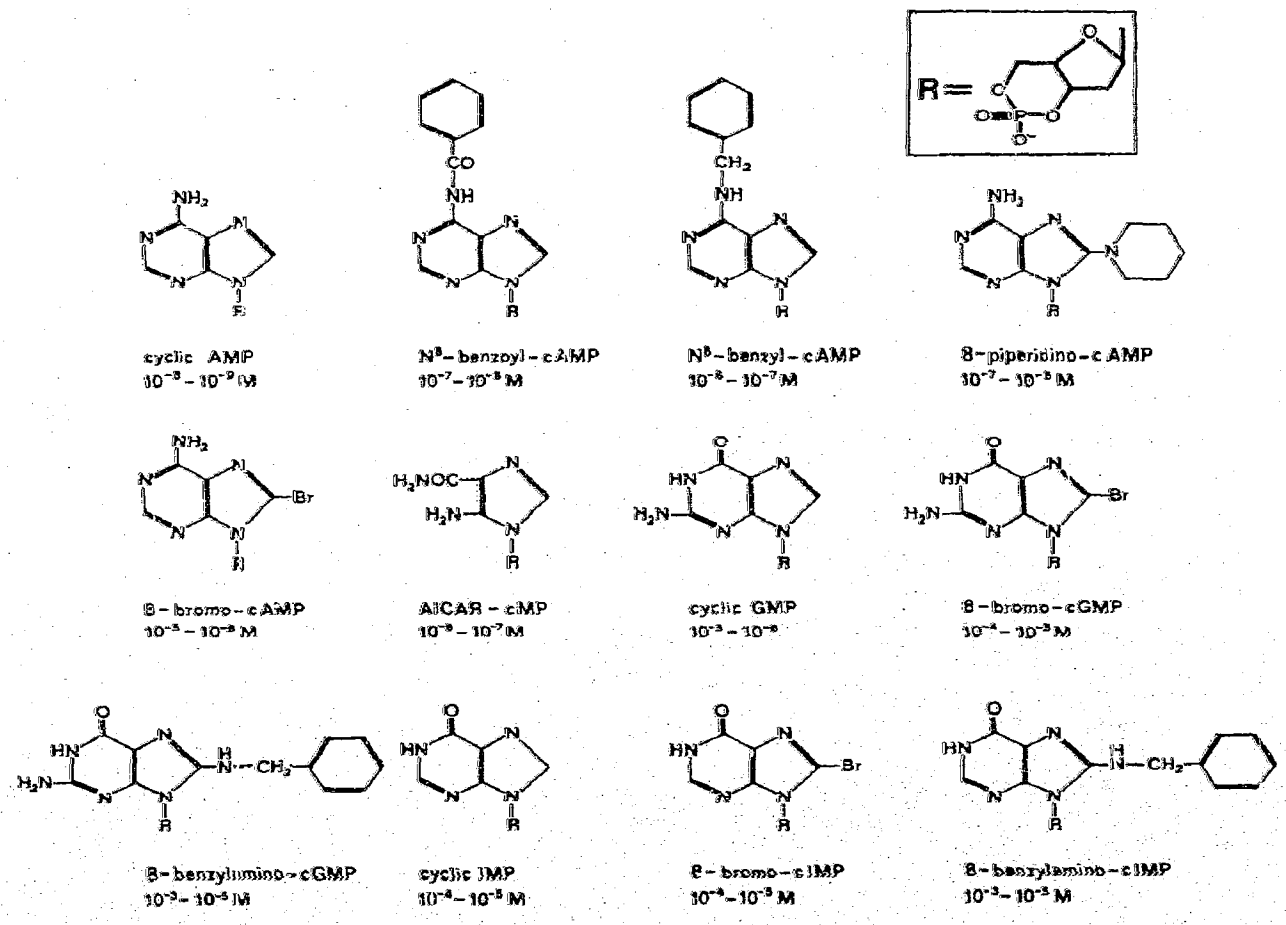

Fig. 1. Chemotactic threshold activities of cyclic nucleotides with substitutions in the base ring.

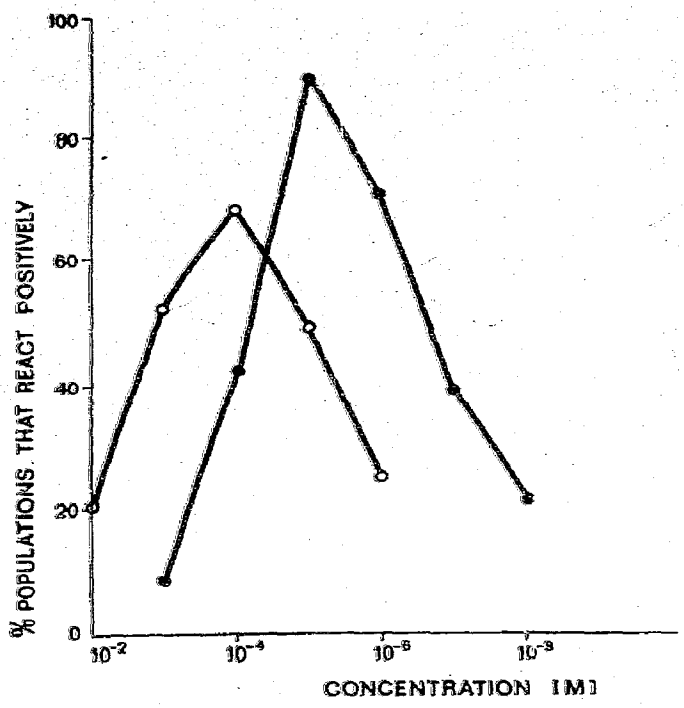

Fig. 2. Per cent of populations of amoebae that react positively at different concentrations of two analogues with substitutions in the base moiety. (•—•—•) N^6-benzyl-cyclic AMP. (o—o—o) 8-Bromo-cyclic GMP.

Cyclic IMP belongs, despite its purine base to the cyclic nucleotides with the lowest chemotactic activity [4]. The same substitutions were introduced at the 8-position of cyclic IMP as were introduced before in cyclic GMP and again the chemotactic activity of the 8-bromo-cyclic IMP was higher than the activity of 8-benzylamino-cyclic IMP (fig. 1).

The influence of these analogues of cyclic nucleotides on phosphorylase *b* kinase activation had been tested in a liver extract [5]. The concentrations of the 8-substituted analogues needed for phosphorylase *b* kinase activation showed some correlation with the threshold activities of these analogues for chemotactic activity. No correlation, however, has been found if 6-substituted analogues were used in both systems.

Each analogue had its specific optimal concentration to attract amoebae. At still higher concentrations the amoebae were not able to detect the gradient of the analogue and the chemotactic response diminished (fig. 2).

The optimal response of the amoebae to the cyclic nucleotide or its analogue was generally observed 5 min after the last deposition of the compound. After 15 min less amoebae populations reacted positively.

Analogues at high concentrations delay and at very high concentrations prevent cell aggregation. 8-Bromo-cyclic GMP at a concentration of 10^{-2} M allowed only a small part of the amoebae populations to aggregate. The inhibition has most likely not been caused by the aspecific effect of a high concentration which would have harmed the amoebae independent of the kind of molecule. Even at high concentrations of the analogue only a relatively few molecules, contained in the 0.1 µl drop, will reach the amoebae and the bulk of the molecules diffuse into other directions and into the agar.

8-Bromo-cyclic AMP, a chemotactically more active substance than 8-bromo-cyclic GMP inhibited aggregation completely at a concentration of 10^{-4} M. The even more active 3-piperidino-cyclic AMP may prevent aggregation at a concentration of 10^{-5} M. The concentrations of the analogues at which aggregation is prevented is correlated to their threshold activities.

Apparently the receptor mechanisms has been oversaturated at these high concentrations of the analogues and amoebae were not able any more to react to the attractant secreted by neighbouring cells.

These, and studies previously described [4] would indicate that substitution in the base of the cyclic nucleotide may change drastically its chemotactic effect and consequently the aggregative behavior of the amoebae.

It is not possible yet to predict the effect of a chemical modification in the adenine base on the interaction between the molecule and the receptor system of the amoebae.

Acknowledgements

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