# STIMULATION OF ADENOSINE 3',5'-MONOPHOSPHATE-DEPENDENT AND GUANOSINE 3',5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASES BY SOME ANALOGS OF ADENOSINE 3',5'-MONOPHOSPHATE

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Summary:

The effect of some analogs of adenosine 3',5'-monophosphate (cyclic AMP) on the activity of protein kinases activated specifically either by cyclic AMP or by guanosine 3',5'-monophosphate (cyclic GMP) has been examined. Tubercidin 3',5'-monophosphate (cyclic TuMP) stimulated the activity of cyclic AMP-dependent protein kinases as effectively as cyclic AMP. The ability of cyclic TuMP to stimulate cyclic GMP-dependent protein kinases was intermediate between that of cyclic GMP and cyclic AMP. The 5'-methylene cyclic phosphonate analog of cyclic AMP caused varying degrees of activation of different cyclic AMP-dependent protein kinases, but was inactive with cyclic GMP-dependent enzymes. The 3'-methylene cyclic phosphonate analog of cyclic AMP was inactive with enzymes of either class.

Cyclic 3',5'-nucleotide-dependent protein kinases which catalyze the phosphorylation of casein, protamine, and histone by ATP have recently been found in a wide variety of tissues throughout the animal kingdom (1-6). Some of these protein kinases are activated by low concentrations of cyclic AMP (1-5) and others by low concentrations of cyclic GMP (6). In view of the possibility (4,6) that the various, diverse effects of cyclic AMP and cyclic GMP may be mediated through regulation of the activity of these protein kinases, it seemed desirable for several reasons to study the effects on these enzymes of compounds structurally related to cyclic AMP and cyclic GMP. Studies with such analogs may increase our understanding of the nature of the interaction between the cyclic nucleotide and the

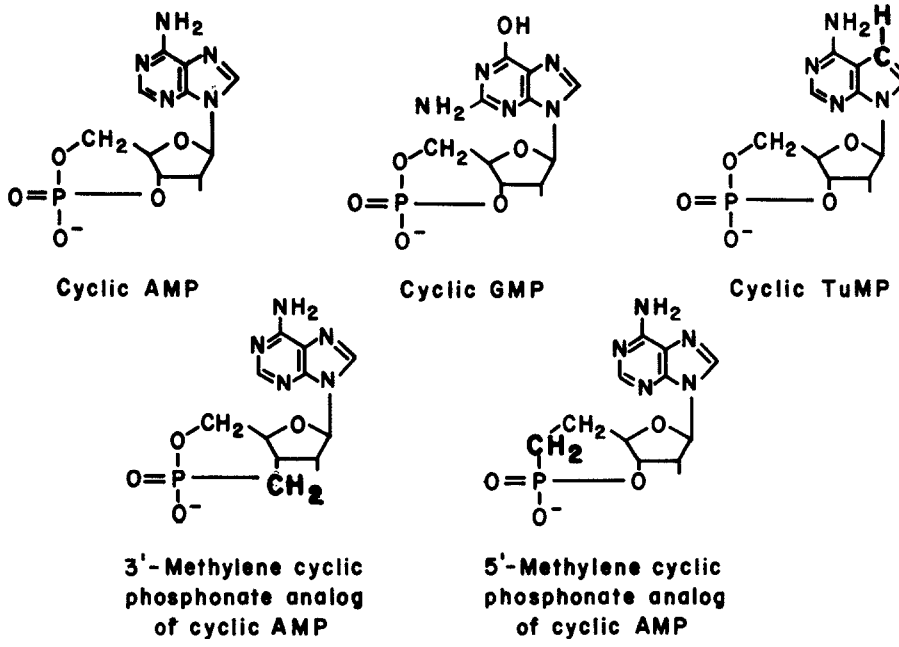


**Fig. 1 Structures of cyclic 3', 5'-nucleotides**

enzyme, help to reveal significant structural and kinetic differences among the various protein kinases, and lay the groundwork for the development of pharmacological agents which would either mimic or antagonize the actions of the cyclic nucleotides, and thereby regulate the activities of these potentially key enzymes.

The structural analogs of cyclic AMP studied in the present investigation were tubercidin 3',5'-monophosphate (cyclic TuMP), the 5'-methylene cyclic phosphonate analog of cyclic AMP and the 3'-methylene cyclic phosphonate analog of cyclic AMP. Their structures, together with those of cyclic AMP and cyclic GMP, are shown in Fig. 1.

Materials and Methods: The cyclic AMP-dependent protein kinases from bovine brain and heart were purified to the stage of DEAE-cellulose chromatography (3,5), and from rat isolated adipose cells to the stage of ammonium sulfate fractionation (4). Cyclic AMP-dependent and cyclic GMP-dependent protein kinases from lobster tail muscle were purified and separated from

each other by DEAE-cellulose column chromotography as previously reported (6). Cyclic AMP was purchased from Schwarz BioResearch, and cyclic GMP from Boehringer-Mannheim. $\gamma^{32}$P-ATP was prepared by the method of Post and Sen (7). Cyclic TuMP was the kind gift of Dr. A. R. Hanze, Upjohn Co.; the 3'-methylene cyclic phosphonate and 5'-methylene cyclic phosphonate analogs of cyclic AMP were generously supplied by Dr. J. G. Moffatt, Syntex Institute of Molecular Biology. Histone (mixture) was purchased from Mann Research Labs.

Protein kinase activity was assayed by measuring the phosphorylation of histone according to a recent modification (8) of methods previously employed (3-5). The incubation mixture contained, in a final volume of 0.2 ml, sodium acetate buffer, pH 6.0, 10 μmole; histone mixture, 40 μg; $\gamma^{32}$P-ATP, 2.5 mμmole, containing about 2.5 x $10^6$ cpm; magnesium acetate, 2 μmole; cyclic nucleotide as indicated; cyclic nucleotide-dependent protein kinase, 3 μg to 21 μg. One unit of protein kinase activity is defined as that amount of enzyme that transferred 1 pmole ($10^{-12}$ mole) of $^{32}$P from $\gamma^{32}$P-ATP to recovered protein in 5 min at 30° in the standard assay system.

Results and Discussion: Tables 1 and 2 compare the ability of cyclic AMP, cyclic GMP, and various analogs of cyclic AMP, to activate cyclic AMP-dependent and cyclic GMP-dependent protein kinases from various sources. In optimal concentrations, cyclic TuMP was able to activate maximally both the cyclic AMP-dependent and the cyclic GMP-dependent enzymes. Moreover, the apparent $K_a$ value (the concentration of cyclic nucleotide required to give a half-maximal increase in activity) of each of the cyclic AMP-dependent enzymes for cyclic TuMP was similar to that of the enzyme for cyclic AMP itself, and 10- to 200-fold lower than for cyclic GMP. The apparent $K_a$ of the cyclic GMP-dependent enzyme for cyclic TuMP was lower than that for cyclic AMP, although greater than that for cyclic GMP. Cyclic TuMP also had an apparent $K_a$ value intermediate between those for cyclic AMP and cyclic GMP when these compounds were tested with a cyclic GMP-dependent protein kinase

TABLE I. Stimulation by cyclic AMP analogs of cyclic AMP-dependent and cyclic GMP-dependent protein kinases.

| Cyclic nucleotide (M) | Enzyme Source and Units of Activity | | | | |
|---|---|---|---|---|---|
| | Bovine brain* | Bovine heart* | Rat adipose cells* | Lobster muscle* | Lobster muscle+ |
| None | 8.6 | 30.1 | 9.6 | 13.4 | 12.2 |
| Cyclic TuMP | | | | | |
| $5.0 \times 10^{-10}$ | 9.5 | 44.5 | 11.1 | 15.9 | 13.7 |
| $5.0 \times 10^{-9}$ | 11.4 | 48.0 | 14.6 | 18.7 | 13.2 |
| $5.0 \times 10^{-8}$ | 31.8 | 101.6 | 16.2 | 36.1 | 17.9 |
| $5.0 \times 10^{-7}$ | 106.7 | 146.1 | 37.6 | 43.4 | 27.3 |
| $5.0 \times 10^{-6}$ | 131.4 | 141.2 | 38.5 | 43.9 | 42.5 |
| $5.0 \times 10^{-5}$ | 126.7 | 119.9 | 34.7 | 42.2 | 46.0 |
| $5.0 \times 10^{-4}$ | 61.7 | 62.4 | 15.7 | 25.9 | 20.3 |
| 5'-methylene analog of cyclic AMP | | | | | |
| $5 \times 10^{-8}$ | 10.2 | 33.6 | 10.5 | 14.9 | 12.2 |
| $5 \times 10^{-7}$ | 12.1 | 71.5 | 11.1 | 29.6 | 12.9 |
| $5 \times 10^{-6}$ | 27.9 | 142.3 | 17.1 | 35.2 | 13.3 |
| $5 \times 10^{-5}$ | 61.5 | 155.7 | 29.3 | 42.1 | 13.2 |
| 3'-methylene analog of cyclic AMP | | | | | |
| $5.0 \times 10^{-6}$ | 9.0 | 35.1 | 8.7 | 11.7 | 11.7 |
| $5.0 \times 10^{-5}$ | 10.0 | 42.4 | 7.8 | 13.8 | 11.5 |
| Cyclic AMP | | | | | |
| $5.0 \times 10^{-7}$ | 126.2 | 140.9 | 35.6 | 48.4 | 17.9 |
| $5.0 \times 10^{-6}$ | 135.4 | 142.2 | 38.1 | 45.6 | 34.1 |
| Cyclic GMP | | | | | |
| $5.0 \times 10^{-7}$ | 18.9 | 52.4 | 24.4 | 24.9 | 35.1 |
| $5.0 \times 10^{-6}$ | 85.9 | 124.3 | 31.8 | 39.8 | 43.7 |

*Cyclic AMP-dependent protein kinase
+Cyclic GMP-dependent protein kinase

recently purified from the larval body wall of the cecropia silk-worm (J. F. Kuo, G. R. Wyatt and P. Greengard, in preparation).

The 5'-methylene cyclic phosphonate analog of cyclic AMP was also able, in optimal concentrations, to activate the various cyclic AMP-dependent

TABLE II. Comparison of apparent $K_a$ values for cyclic nucleotides.

| Sources of protein kinases | Cyclic nucleotide specificity | Apparent $K_a$* (μM) | | | |
|---|---|---|---|---|---|
| | | Cyclic AMP | Cyclic GMP | Cyclic TuMP | 5'-$CH_2$-Cyclic AMP |
| Bovine brain | Cyclic AMP | 0.110+ | 7.9 | 0.16 | 50.0 |
| Bovine heart | Cyclic AMP | 0.040+ | 5.0 | 0.025 | 1.1 |
| Rat adipocytes | Cyclic AMP | 0.039 | 1.0 | 0.080 | 16.0 |
| Lobster muscle | Cyclic AMP | 0.018‡ | 1.2‡ | 0.016 | 1.2 |
| Lobster muscle | Cyclic GMP | 3.6‡ | 0.075‡ | 0.80 | --- |

*The apparent $K_a$ values have been calculated from dose-response relationships much more detailed than those shown in Table I.

+Taken from Ref. 5; ‡Taken from Ref. 6. The incubation conditions employed previously (5,6) were slightly different from the present ones. However, no significant differences were observed in the apparent $K_a$ values in those instances in which measurements were made using both sets of incubation conditions.

protein kinases to maximal or near maximal levels of activity. However, the apparent $K_a$ values of the cyclic AMP-dependent enzymes for this analog were 25- to 500-fold greater than for cyclic AMP. Interestingly, the analog was effective at much lower concentrations with the cyclic AMP-dependent protein kinases from muscle tissue than it was with the cyclic AMP-dependent protein kinases from the other types of tissue studied. This large variation in apparent $K_a$ value suggests some basic difference in the nature of the protein kinases from various sources, in agreement with certain other differences in the protein kinases from different tissues reported earlier (4,5). The 5'-methylene cyclic phosphonate analog was inactive with the cyclic GMP-dependent enzyme from lobster tail muscle. The 3'-methylene cyclic phosphonate analog of cyclic AMP showed little or no ability to activate enzymes either of the cyclic AMP-dependent or of the cyclic GMP-dependent class. Both the 3'-methylene and the 5'-methylene cyclic phosphonate analogs

of cyclic AMP were also without any detectable effect on the cyclic GMP-dependent protein kinase from the cecropia silk-worm (J. F. Kuo, G. R. Wyatt, and P. Greengard, in preparation).

Cyclic TuMP, the 3'-methylene and the 5'-methylene cyclic phosphonate analogs of cyclic AMP were tested for their ability to influence the activation, by suboptimal amounts of cyclic AMP, of the cyclic AMP-dependent enzymes from bovine brain, bovine heart and rat adipocytes. No evidence of inhibitory activity was found for any of the three compounds with any of the enzymes studied. The combination of a suboptimal amount of cyclic AMP plus a suboptimal amount of cyclic TuMP or of the 5'-methylene cyclic phosphonate analog of cyclic AMP showed additive effects on the activity of the enzyme.

Butcher and Sutherland (cited by Hanze (9)) found cyclic TuMP to be as active as cyclic AMP in the liver phosphorylase activation assay and Drummond and Powell (10) found cyclic TuMP to be slightly more active than cyclic AMP in activating phosphorylase b kinase from skeletal muscle. The present demonstration of the ability of cyclic TuMP to activate various cyclic AMP-dependent protein kinases as effectively as cyclic AMP provides an explanation for the earlier findings, since it now seems very likely (1) that the activation by cyclic AMP of phosphorylase kinase and phosphorylase is mediated through cyclic AMP-dependent protein kinase activity.

The effectiveness of cyclic TuMP in activating cyclic AMP-dependent and cyclic GMP-dependent protein kinases from various sources and the selective activation of cyclic AMP-dependent protein kinases from muscle by low concentrations of the 5'-methylene cyclic phosphonate analog of cyclic AMP encourage the hope that potent pharmacological agents will be found which, through activation or inhibition, can regulate the activity of tissue-specific cyclic nucleotide-dependent protein kinases.

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