ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE DERIVATIVES

II. BIOLOGICAL ACTIVITY OF SOME 8-SUBSTITUTED ANALOGS

Randy J. Bauer, Kenneth R. Swiatek, Roland K. Robins, and Lionel N. Simon*

ICN Nucleic Acid Research Institute, Irvine, CA 92664 and

the Illinois State Pediatric Institute, Chicago, IL 60608

Received August 19, 1971

SUMMARY

Several 8-substituted derivatives of adenosine 3',5'-cyclic monophosphate have been shown to stimulate the ATP-dependent phosphorylation of histone by purified bovine brain and liver protein kinase as well as to stimulate glycogenolysis in rat liver slices. The results indicate that a series of 8-substituted analogs which have varying activation constants (Ka values) for the bovine kinases, have the same order of relative potency as stimulators of glycogenolysis in liver slices.

INTRODUCTION

Attempts to correlate structural modifications and biological activity have awaited the synthesis of a group of analogs whose chemical structure is varied in a regular fashion. In a previous report, Muneyama et al. (1), (paper I of this series) have synthesized derivatives of adenosine 3',5'-cyclic monophosphate (cAMP) substituted in the 8 position which stimulate ATP-dependent phosphorylation of histone by a purified protein kinase isolated from bovine brain. Michal et al. (2) have shown that a series of cAMP derivatives mainly bearing an 8-substituted amino function were capable of stimulating phosphorylase activation in rat liver homogenates. Cehovic et al. (3) have shown that several analogs of cAMP were capable in vitro of stimulating the release of growth hormone and prolactin from the pituitary gland, but none of these studies have dealt with structure activity relationships of both the purified protein kinase and whole cell systems.

The purpose of this paper is to report the activation constants (Ka

* Reprints: Dr. L. N. Simon, ICN Nucleic Acid Research Institute, 2727 Campus Drive, Irvine, CA 92664.

values) of a series of 8-substituted cAMP analogs for bovine brain and rat liver protein kinase and furthermore to show that the order of relative activity (α) of these compounds is similar to their order of potency as stimulators of rat liver glycogenolysis.

METHODS

(γ - ^{32}P) ATP was obtained from International Chemical and Nuclear Corp. Analogs of cAMP were prepared by the method of Muneyama et al. (1) and were chromatographically and analytically pure. Bovine brain and liver protein kinase were purified to the stage of DEAE-cellulose chromatography using the procedure of Miyamoto et al. (4). Liver was removed from Sprague-Dawley rats and 50-70 mg slices were cut by hand and incubated for the times indicated in 4 ml of Krebs-Henseleit buffer (5), pH 7.4, at 40° with O_2 + CO_2 (95:5) in the gas phase. At the end of the incubation, 0.1 vol. of 3N perchloric acid was added to stop the reaction and the mixture worked up and glucose determined as previously described (6).

RESULTS AND DISCUSSION

Our previous results (1) have shown that the 8-thio derivatives of cAMP were by and large better activators of bovine brain protein kinase than the 8-oxo which in turn were better than the 8-amino derivatives of cAMP. The results presented in Table I indicate that based on relative Ka' values (Ka' = Ka cAMP/Ka test compound), the 8-thio > 8-oxo > 8-amino in activating the protein kinase from the liver as well as the brain. Furthermore, 8-(2-hydroxyethylamino)cAMP was found to be about one-fourth as active as the 8-amino derivative in activating the liver protein kinase and also about one-fourth as active in stimulating glycogenolysis in rat liver slices. Although the quantitative values for the Ka' varied somewhat for the two tissues, the order of potency of these derivatives was the same for the two enzyme systems.

Since a variation in activity due to functional groups at the 8 position of cAMP was found for the isolated enzyme system, we examined whether a similar order of potency existed in whole cell systems. The results shown in

Table I

Ka and Ka' Values for Several 8-Substituted cAMP Analogs

	Liver		Brain	
8-R =	Ka x 10^8	Ka'*	Ka x 10^8	Ka'*
-H	2.54	-	4.83	-
$-SCH_2C_6H_5$	1.06	2.39	2.76	1.75
-SH	1.21	2.1	1.28	3.78
-OH	1.68	1.51	1.77	2.78
$-NH_2$	4.87	0.52	3.15	1.54
$-NHCH_2CH_2OH$	16.40	0.155	27.60	0.175
$-NHCH_2C_6H_5$	16.50	0.154	58.10	0.083

* Ka' = Ka cAMP/Ka test compound

Table II indicate that an A_{50} value determined for these same compounds over a concentration range of 1 x 10^{-4} to 2 x 10^{-8} M was surprisingly similar to the order of potency obtained for the purified protein kinases shown in Table I. All of the measurements for determining A_{50} values were made after 90 minutes of incubation.

The results shown in Figures 1a and 1b show a time course for several of the compounds studied at two different concentrations. Several interesting points should be noticed from these graphs. First, at high substrate concentration (1 x 10^{-3} M) all the compounds studied appeared to stimulate glycogenolysis with the single exception of 8-thio-cAMP. Secondly, cAMP itself at 1 x 10^{-5} was completely inactive in this whole cell system. The lack of activity of cAMP could be attributed to its relative inability to penetrate the cell or to its destruction by cAMP phosphodiesterases in this preparation. Similarly, the enhanced activity of these 8-substituted analogs as compared to cAMP at low substrate concentration might be explainable by virtue of

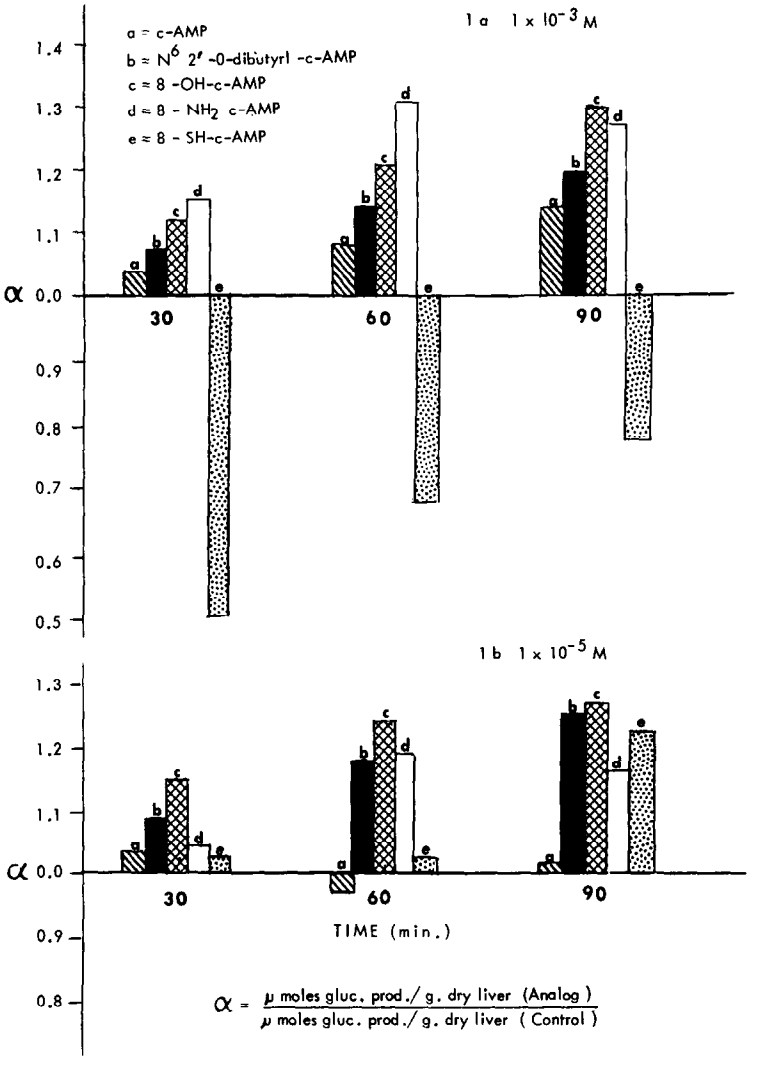

Figure 1. The relative potency (α) of several cAMP analogs at 1 x 10^{-3} M (1a) and 1 x 10^{-5} M (1b) on glucose production in rat liver slices with time.

increased penetrability into the cell or a decreased rate of destruction by phosphodiesterase. It has already been shown that 8-amino-cAMP is degraded at a rate approximately 80% of that observed for cAMP (1). For this reason we feel that the enhanced activity of these 8-substituted derivatives in the liver slice system is probably due to increased penetration into the cell, coupled with the fact that they are potent activators of the liver protein kinase.

Table II

A_{50} Values for Several cAMP Analogs Determined by Glucose Production in Rat Liver Slices

Analog	$A_{50} \times 10^{-6}$ M
cAMP	100.0
N^6,2'-O-dibutryl-cAMP	5.0
8-SH-cAMP	1.0
8-OH-cAMP	1.28
8-NH_2-cAMP	4.60
8-$SCH_2C_6H_5$-cAMP	2.66
8-SCH_3-cAMP	2.82
8-SCH_2CH_2OH-cAMP	3.50
8-SCH_2CH_3-cAMP	3.90
8-$NHCH_2CH_2OH$-cAMP	15.80

Finally, the finding of lower free glucose levels in the 8-thio-cAMP treated slices at 1×10^{-3} M than in control slices may be explainable in one of the following ways. First, glucose utilization via the hexose monophosphate shunt or glycolysis may be stimulated or secondly, glycogen synthesis rather than breakdown may also be stimulated and/or, a combination of these processes. In addition, the decrease in inhibition observed with time may be due to conversion of the 8-thio analog to 8-methylthio-cAMP which we have shown to stimulate glycogenolysis at 1×10^{-3} M (Swiatek, unpublished observation) or removal via some other pathway resulting in lower tissue concentration of this derivative. At 1×10^{-5} M, 8-thio-cAMP stimulated glycogenolysis Each of these various possibilities is currently being investigated.

ACKNOWLDEDGEMENT

This investigation was supported in part by research grants from the United States Public Health Service AM-14363-01 and AM-14363-02.

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