CYCLIC GMP STIMULATION AND INHIBITION OF CYCLIC AMP PHOSPHODIESTERASE FROM THYMIC LYMPHOCYTES*

D. J. FRANKS** and J. P. MACMANUS

Division of Biology, National Research Council of Canada, Ottawa 7, Canada

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SUMMARY. A particulate preparation of cyclic AMP phosphodiesterase from rat thymic lymphocytes exhibited two apparent Km's at 0.9×10^{-6}M and 8.0×10^{-6}M. The enzyme with the higher Km was stimulated by cyclic GMP by a mechanism involving an increase in the Vmax of the enzyme with no change in the Km. Cyclic GMP competitively inhibited the enzyme with the low apparent Km which had a Ki for cyclic GMP of 4×10^{-5}M. The modulation of cyclic AMP phosphodiesterase activity by cyclic GMP in the control of cyclic AMP-mediated lymphocyte proliferation is discussed.

INTRODUCTION

Low concentrations of exogenous cyclic AMP stimulate the proliferation of a variety of cells (1-4) and the cyclic nucleotide is probably involved in the control of cell proliferation. High concentrations (5×10^{-6}M) of exogenous cyclic GMP have been reported to mimic this effect of exogenous cyclic AMP on thymic lymphocyte proliferation (5). However, lower concentrations of cyclic GMP (10^{-7} - 10^{-9}M) are without effect on cell proliferation (5) but they do completely block the mitogenic effect of exogenous cyclic AMP (6).

High concentrations of cyclic GMP have been consistently shown to inhibit cyclic AMP phosphodiesterase (5, 7-9) and thus to increase the cellular content of cyclic AMP (5-7). However, a recent report indicates that under certain conditions cyclic GMP can also stimulate the hydrolysis of

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cyclic AMP (9). Thus, depending on the concentration of exogenous cyclic GMP, the cellular content of cyclic AMP may be controlled by cyclic GMP-induced changes in the activity of cyclic AMP phosphodiesterase.

Since two opposite effects of cyclic GMP on cell proliferation can be observed using thymic lymphocytes, it is possible that two opposite effects of this cyclic nucleotide on cyclic AMP phosphodiesterase activity can also be demonstrated.

MATERIALS AND METHODS

Rat thymic lymphocytes were isolated (10) and the cell suspension centrifuged at 500 x g for 10 min. The packed cells were resuspended in 9 volumes of 60mM Tris buffer (pH 7.5) homogenized at 16,000 rpm for 2 min in a Sorvall Omnimixer, and then centrifuged at 20,000 x g for 10 min. The pellet was resuspended in 60mM Tris buffer (pH 7.5) and stored at 4°C prior to use.

Cyclic AMP phosphodiesterase (cyclic AMP-PDE) was assayed by the method of Brooker et al. (11). Enzyme concentration was adjusted so that the cyclic AMP hydrolysis was less than 30% at any substrate concentration. Protein was determined by the method of Lowry et al. (12) using bovine serum albumin as the standard.

^{3}H-cyclic AMP (3.0 Ci/mmole) was purchased from Amersham-Searle, Chicago, Illinois. Snake venom (Ophiophagus hanna) 3'5' cyclic GMP, 2'3' cyclic GMP and 5' GMP were purchased from Sigma Chemical Co., and 3'5' cyclic AMP from Schwartz-Mann Inc.

RESULTS AND DISCUSSION

The cyclic AMP-PDE activity in the 20,000 x g pellet was linear both with increasing enzyme concentration and time. It should also be noted that the snake venom used in excess in the assay was capable of hydrolyzing

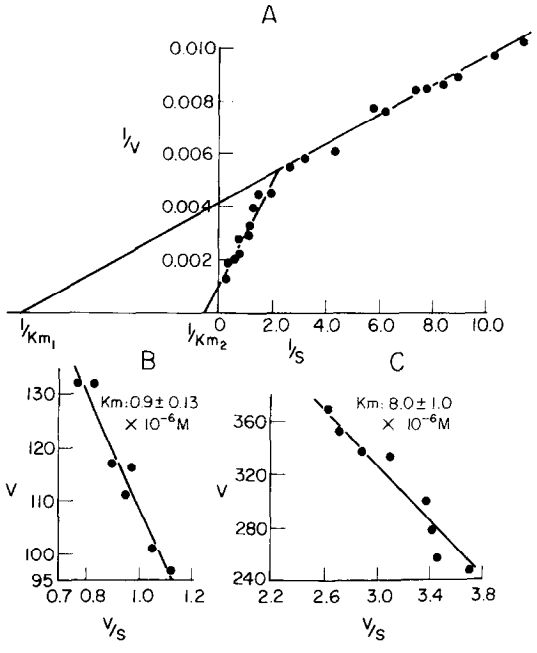

Fig. 1. A. Lineweaver-Burke plot of activity of cyclic AMP-PDE when the cyclic AMP substrate concentration was between 5×10^{-7} M and 1×10^{-5} M. B and C show Hofstee plots for the determination of the apparent Km's when the substrate concentration was between 5×10^{-7} M and 1×10^{-6} M and between 5×10^{-6} M and 1×10^{-5} M. Velocity is expressed as pmoles cyclic AMP hydrolyzed/mg protein/10 min.

100% of the 5' AMP produced from cyclic AMP and itself had no cyclic AMP-PDE activity.

When cyclic AMP-PDE activity was assayed over a wide range of substrate concentrations the Lineweaver-Burke plot was found to be non-linear (Fig. 1A). These observations suggested that the cyclic AMP-PDE might have more than one Km. Furthermore, this plot gave an approximate estimation of the value of the apparent Km's if it was represented as two intersecting straight lines (Fig. 1A). In order to accurately determine their values, the enzyme was assayed at substrate concentrations close to the two apparent Km's (Fig. 1B and C) and values of $0.9 \pm 0.13 \times 10^{-6}$ M and $8.0 \pm 1.0 \times 10^{-6}$ M were obtained.

Concentrations of cyclic GMP up to 10^{-4}M progressively inhibited cyclic AMP hydrolysis when the substrate concentration was 10^{-6}M (Fig. 2A). Over 60% inhibition of cyclic AMP hydrolysis was found in the presence of 10^{-4}M cyclic GMP. 5' AMP did not inhibit the enzyme at any concentration but 2'3' cyclic GMP did cause 15% inhibition when present at 10^{-4}M (data not shown).

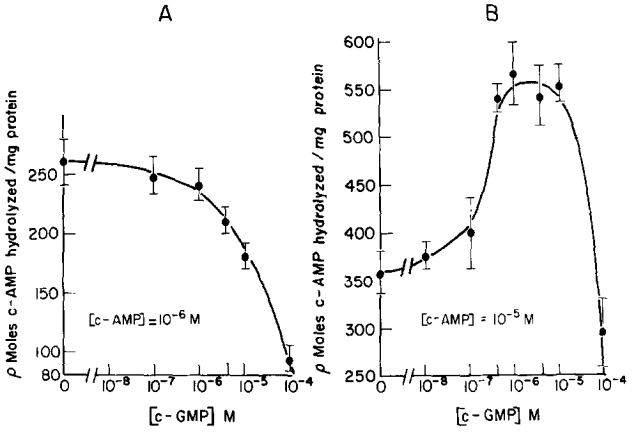

Fig. 2. A. Effect of cyclic GMP on cyclic AMP-PDE activity in the presence of $1x10^{-6}$M cyclic AMP. B. Effect of cyclic GMP on cyclic AMP-PDE activity in the presence of $1x10^{-5}$M cyclic AMP.

At a substrate concentration of 10^{-5}M, cyclic GMP stimulated cyclic AMP-PDE activity (Fig. 2B). The maximum stimulation (65%; $p < 0.001$) occurred between $5x10^{-7}$M and $1x10^{-5}$M cyclic GMP. No stimulation could be observed using either 5' GMP or 2'3' cyclic GMP.

The greatest inhibition of cyclic AMP-PDE activity by cyclic GMP occurred in the presence of low cyclic AMP concentrations (Fig. 2A and B) which suggested that the inhibition was a property of the low Km enzyme. The inhibition was found to be competitive with a Ki for cyclic GMP of $4x10^{-5}$M (Fig. 3).

The stimulation of cyclic AMP hydrolysis by cyclic GMP

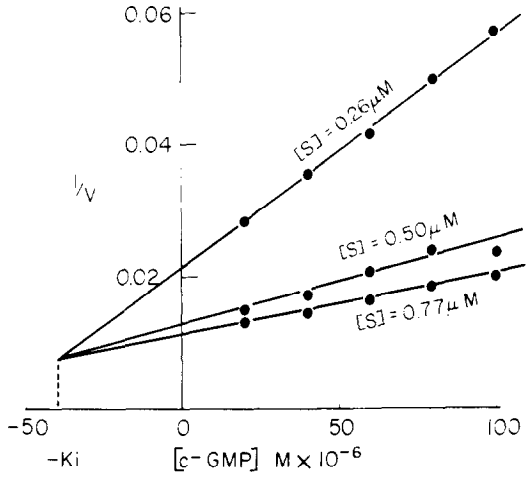

Fig. 3. Dixon plot to determine the Ki for cyclic GMP of cyclic AMP-PDE activity. [S] is the concentration of cyclic AMP. Velocity is expressed as pmoles cyclic AMP hydrolyzed/mg protein/10 min.

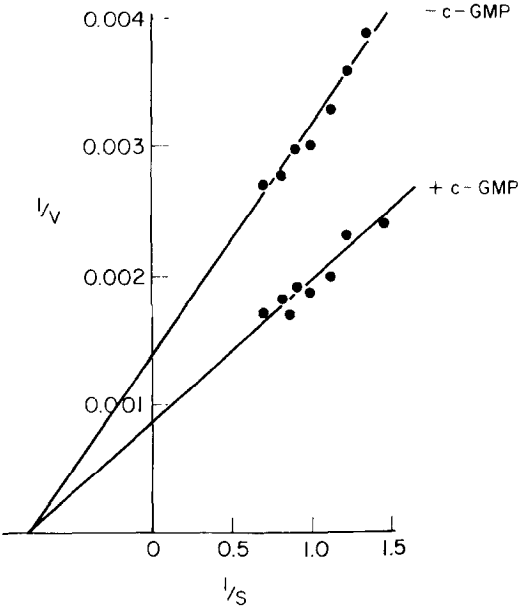

Fig. 4. Lineweaver-Burke plot of cyclic AMP-PDE activity showing the effect of $1x10^{-6}$M cyclic GMP. The concentration of cyclic AMP ranged from $4x10^{-6}$M to $1x10^{-5}$M. Velocity is expressed as pmoles cyclic AMP hydrolyzed/mg protein/10 min.

appeared to be a property of the higher Km enzyme. Fig. 4 shows a Lineweaver Burke plot which indicated that no change in the Km could be detected in the presence of 10^{-6}M cyclic GMP but the Vmax increased significantly from 681 ± 48 to 1111 ± 150 pmoles cyclic AMP hydrolyzed/mg protein/10 min ($p < 0.001$).

These results indicate that cyclic GMP could play an important role in the control of the cyclic AMP concentration in thymic lymphocytes by modulating the hydrolysis of cyclic AMP. Such a concentration-dependent dual action of cyclic GMP on cyclic AMP-PDE activity could account for the opposite effects of high and low concentrations of exogenous cyclic GMP on cell proliferation (5).

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