Prostaglandin Regulation of Adenylate Kinases Purified from Liver, Skeletal Muscle, and Hepatoma

Supported in part by USPHS grant CA11818. WEC is a Research Career Development Awardee, CA70187

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Key Words

Prostaglandins A₁, A₂, E₁, E₂, F_{2α}
Stimulation and inhibition of rat liver adenylate kinase
Skeletal muscle
Morris hepatoma 3924 A

Abstract

Adenylate kinases, purified from adult rat liver and skeletal muscle and from a fast growing hepatoma, were examined in the presence of a series of prostaglandins. Prostaglandins A₁, A₂, E₁, E₂, and F_{2α} stimulated liver adenylate kinase 27% to 149%. All of these prostaglandins stimulated the skeletal muscle enzyme from 47% to 82%. While prostaglandins A₂ and E₂ stimulated, prostaglandins A₁ and F_{2α} inhibited, and prostaglandin E₁ was without effect on the hepatoma adenylate kinase.

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Introduction

Prostaglandins are a group of pharmacologically and physiologically active lipids which are widely distributed in most mammalian tissues and body fluids. They are directly involved in the relaxation-contraction of smooth muscle, gastric acid secretion, platelet aggregation, luteolysis, etc. [1]. Evidence has also been presented that prostaglandin action may involve cyclic AMP or the activity of adenylate cyclase. Altered responsiveness of tumor adenylate cyclases to prostaglandin action has been observed in HeLa cells and HTC cells [2], Ehrlich ascites cells [3], normal versus transformed BHK cells [4], and in Morris hepatoma 5123TC(h) [5] and 3924A [6] versus normal liver. Because ATP is the only substrate for adenylate cyclase, and because adenylate kinase is a key homeostatic regulator of ATP levels in liver cells, we have examined the effects of a series of prostaglandins upon the purified adenylate kinases from rat liver, skeletal muscle, and Morris hepatoma 3924A.

Materials and Methods

Adenylate kinase isozymes from rat liver, skeletal muscle, and Morris hepatoma 3924 A were identified and purified according to previously published methods [7, 8, 9]. The final specific activities of liver, skeletal muscle, and tumor enzymes were 670, 1200, and 290 $\mu\text{mol ADP}$ produced per min per mg protein, respectively. Electrophoresis of each of the purified enzymes by polyacrylamide disc gel electrophoresis revealed only one protein band for each enzyme. Sedimentation equilibrium studies by analytical ultracentrifugation gave one peak for each enzyme. The general kinetic and physical protein properties of these three adenylate kinases have been previously reviewed [7].

The assay system routinely consisted of 50 mM triethanol-amine-HCl buffer at pH 7.2, 15 mM ATP, 10 mM AMP, and 15 mM MgCl₂ in one ml final assay volume. Because of the high substrate K_M values previously observed for the tumor adenylate kinase, we used 50 mM of ATP, AMP, and MgCl₂ in the assay for this enzyme. Prostaglandins were dissolved in 95 % ethanol and added in 10 μ l aliquots. All controls contained 10 μ l of 95 % ethanol. The reaction was started by adding 10 μ l of the purified enzyme. The reaction was quenched by addition of 1 ml of cold 1.5 N perchloric acid and immersion into ice. 100 μ l of the reaction mixture was spotted on Whatmann 3 MM filter paper and the adenine nucleotides were separated by high voltage electrophoresis. Nucleotide spots were cut from the filter paper, eluted with 0.75 ammonium bicarbonate, and quantitated by spectrophotometry [9].

Results

Prostaglandins A_i, A₂, E_i, E₂, and F_{2a} stimulated liver adenylate kinase from 27 % to 149 % (table I). These prostaglandins increased the activity of the skeletal muscle enzyme from 47 % to 82 %. While prostaglandins A₂ and E₂ stimulated the tumor adenylate kinase, prostaglandins A_i and F_{2a} were inhibitory, and E_i had no effect on the tumor enzyme.

The apparent Michaelis-Menton constants reflect the activation studies. K_M values from prostaglandins A₂, E_i, and E₂, and F_{2a} ranged from 16 to 35 μ M, 27 to 61 μ M, and 46 to 100 μ M for the liver, skeletal muscle, and hepatoma adenylate kinases, respectively (table II).

Table I. Effects of prostaglandins on the activity of adenylate kinases purified from rat liver, skeletal muscle, and Morris hepatoma 3924 A.

Prostaglandins (10⁻⁴M) Enzymatic activity
(% $\pm$ prostaglandin)

Liver	Hepatoma	Sk. muscle
A _i	+ 33 $\pm$ 4 -20 $\pm$ 3	+82 $\pm$ 6
A ₂	+149 $\pm$ 10 +11 $\pm$ 2	+80 $\pm$ 5
E _i	+129 $\pm$ 8 -01 $\pm$ 1	+47 $\pm$ 4
E ₂	+ 27 $\pm$ 4 +29 $\pm$ 4	+79 $\pm$ 5
F _{2a}	+148 $\pm$ 9 -13 $\pm$ 2	+54 $\pm$ 6

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Table II. Apparent Michaelis-Menton constants for the various prostaglandins.

Discussion

While all of the various prostaglandins (A_i, A₂, E_i, E₂, F_{2a}) stimulated adenylate kinases from both liver and skeletal muscle, only PGA₂ and PGE₂ stimulated the tumor enzyme, PGA_i, and PGF₂ < x were inhibitory. This data complements previously published results indicating that the hepatoma adenylate kinase is not regulated by citrate acid cycle metabolites as is the adult liver form of the enzyme [7, 10]. Other tumor isozymic forms which have altered post-transcriptional regulatability include pyruvate kinase, hexokinase-gluco-kinase, glycogen synthetase, DNA polymerase, aldolase, and lactate dehydrogenase [10, 11, 12]. There is a definite trend here which implicates loss of regulatory control in cancer and the appearance of isozymic forms of enzymes which are less regulatable than their counterparts in normal adult tissues.

Acknowledgments

We would like to express our sincere thanks to Dr. H. P. Morris, Howard University, Washington, D.C. for the generous supply of hepatoma 3924A and to Mr. Willis Parker for his excellent technical assistance.

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