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EXOGENOUS, BUT NOT ENDOGENOUS, CYCLIC GMP REDUCES HEPATIC PYRUVATE KINASE ACTIVITY

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Summary

We investigated the effects of exogenous cyclic GMP and stimulants of endogenous cyclic GMP accumulation on L-form (hepatic) pyruvate kinase (ATP· pyruvate 2-*O*-phosphotransferase, EC 2.7.1 40) activity in isolated rat hepatocytes. Exogenous cyclic GMP (200 μM) reduced pyruvate kinase activity, but was less potent than exogenous cyclic AMP (50 μM) ($K_1 \simeq 120$ μM vs. 30 μM, respectively), had a slower onset of action (1.0 vs. 0.3 min, respectively) and a less rapid maximal effect (5.0 vs. 1.0 min, respectively). Similar results were noted with dibutyryl cyclic GMP or dibutyryl cyclic AMP. 1.0 μM acetylcholine increased cyclic GMP concentrations in isolated hepatocytes from 233 ± 16 to 447 ± 3 pmol/g cell protein ($P < 0.001$), but did not alter pyruvate kinase activity Similar results were noted with carbamylcholine, NaN_3 or acetylcholine plus eserine sulfate. The results suggest a differential effect of exogenous vs. endogenous cyclic GMP on L-form pyruvate kinase activity, and question the physiological relevance of observations with exogenous cyclic GMP in this system.

Introduction

Exogenous cyclic GMP [1] or cyclic AMP [1—3] inactivates hepatic pyruvate kinase (ATP pyruvate 2-*O*-phosphotransferase, EC 2 7 1 40) activity Endogenous cyclic AMP, formed by glucagon stimulation, also reduces pyruvate kinase activity [1—7]. The effects of substances which increase intracellular concentrations of cyclic GMP on hepatic pyruvate kinase activity have not been evaluated. Since the cyclic AMP-like effects produced by exogenous cyclic

GMP in other tissues have not been replicated by stimulants of endogenous cyclic GMP accumulation [8,9], we investigated the effects of exogenous cyclic nucleotides, cholinergic agents and NaN_3 on pyruvate kinase activity in isolated rat hepatocytes

Materials and Methods

Isolated rat hepatocytes [10] were prepared by a modification of the methods of Veneziale et al. [11]. Ca^{2+}-free Krebs-Ringer bicarbonate buffer [12] was perfused by an Ambec perfusion apparatus (MXB, Inc., Aurora, CO) through fresh liver from male Sprague-Dawley rats, 350—500 g. After 10 min, fresh perfusate with collagenase (Worthington, Type I), 30 mg/100 ml, was perfused. Hepatocytes were isolated as described by Veneziale et al. [11] and resuspended in Krebs buffer with Ca^{2+} [12] and 1.5% gelatin

For study of pyruvate kinase activity, the hepatocytes were incubated for 10 s to 15 min in a shaking, metabolic incubator. Pyruvate kinase activity was measured with 0.3 or 4.0 mM phospho*enol*pyruvate as substrate by the method of van Berkel et al [13]. Activity in nmol pyruvate/ml per min, is expressed as the ratio of activities with subsaturating (0.3 mM) vs. saturating (4.0 mM) concentrations of phospho*enol*pyruvate [6], as determined in pilot experiments.

Generation of cyclic GMP and measurement of cyclic GMP accumulation within isolated hepatocytes were carried out by a modification [14] of the method of Steiner et al. [15]. Supernatants of homogenates from hepatocytes, prepared as described by Tihon et al. [14] were diluted 1 10 with 0.05 M acetate buffer, pH 6.25, and acetylated [16] before assay. Cyclic GMP concentrations were measured by radioimmunoassay [17] with a commercial kit (New England Nuclear, Boston, MA). The protein content of the cell suspensions was determined by the method of Lowry et al. [18], with bovine serum albumin (Fraction V, Sigma Chemical, St. Louis, MO) as the protein standard.

Cyclic nucleotides, gelatin, collagenase, lactate dehydrogenase cholinergic agents, phospho*enol*pyruvate, nicotinic adenine dehydrogenase (NADH), Tris-HCl NaN_3, eserine sulfate and adenosine diphosphate were purchased from Sigma Chemical Company (St. Louis, MO). Trichloroacetic acid was obtained from Mallinckrodt Chemical (Paris, KY).

Results

Effects of exogenous cyclic nucleotides on hepatic pyruvate kinase activity

Both exogenous cyclic AMP and exogenous cyclic GMP reduced pyruvate kinase activity in isolated rat hepatocytes (Fig. 1). Cyclic AMP, $K_1 \simeq 30$ μM, was relatively more potent than cyclic GMP, $K_1 \simeq 110$ μM. Dibutyryl cyclic nucleotides also decreased enzyme activity (Fig. 2). Dibutyryl cyclic AMP, $K_1 \simeq 2.5$ μM, was relatively more potent than dibutyryl cyclic GMP, $K_1 \simeq 6.5$ μM. The inactivation of pyruvate kinase activity by cyclic AMP was more rapid than cyclic GMP Basal pyruvate kinase activity, 0.086 ± 0.004 (ratio of activity with 0 3/4.0 mM phospho*enol*pyruvate) was significantly reduced to 0 064 ± 0 005 ($P = 0$ 001) by cyclic AMP, (50 μM) after 0.3 min incubation, whereas significant reduction ($P < 0.001$) of the enzyme was not noted with cyclic GMP

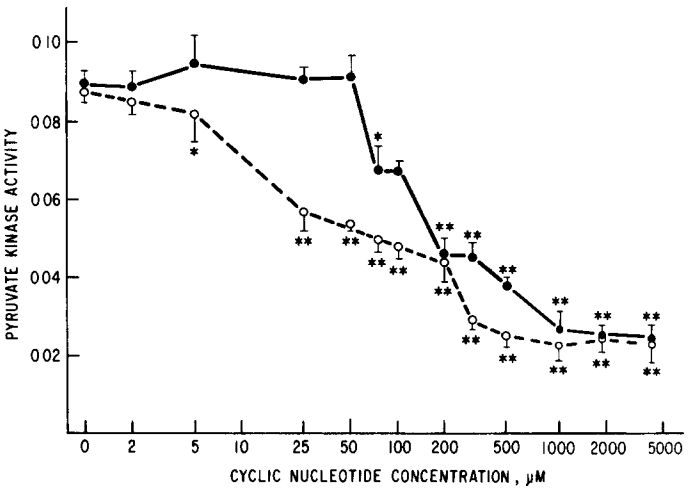

Fig 1 Hepatic pyruvate kinase activity, expressed as the ratio of activities with 0 3 and 4 0 mM phospho*enol*pyruvate, observed with increasing concentrations of cyclic AMP (○ - - - - - - ○) or cyclic GMP (●——●) The concentrations of cyclic nucleotide are plotted on a logarithmic scale *, $P < 0\ 05$ vs control ** $P < 0\ 01$ vs control.

(200 μM) until 1.0 or more min incubation. Maximal reduction was observed with cyclic AMP after 1.0 min incubation, and with cyclic GMP after 5 min incubation. Combinations of submaximal or maximal concentrations of cyclic AMP and cyclic GMP produced no greater decrease in enzyme activity than that observed with cyclic AMP alone (Table I).

Effects of Cholinergic Agents and NaN_3. The effects of acetylcholine, carbamylcholine and NaN_3 on pyruvate kinase and cyclic GMP accumulation were tested. Acetylcholine, 0.1—100 μM, failed to alter pyruvate kinase activity (Table II), however, the same concentrations of acetylcholine significantly increased intracellular levels of cyclic GMP (Table II). NaN_3 0.1—5 mM, also

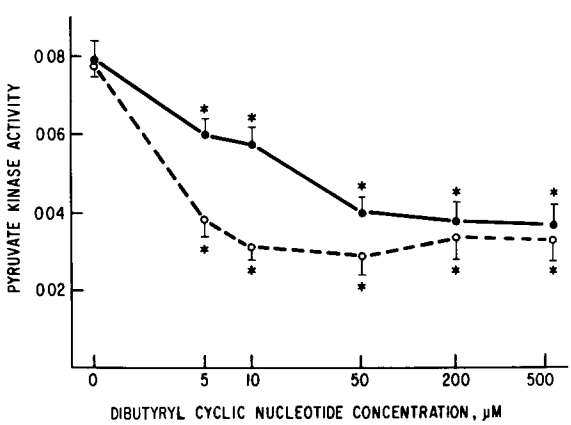

Fig 2 Hepatic pyruvate kinase activity (ratio of activities with 0 3 and 4 0 mM phospho*enol*pyruvate) observed with increasing concentrations of dibutyryl cyclic AMP (○ - - - - - - ○) or dibutyryl cyclic GMP (●——●) * $P < 0\ 01$ vs control

TABLE I

INHIBITION OF L-FORM PYRUVATE KINASE ACTIVITY BY SUBMAXIMAL AND MAXIMAL CONCENTRATIONS OF CYCLIC AMP AND CYCLIC GMP, ALONE OR IN COMBINATION

Hepatocytes were homogenized and centrifuged at 20 000 × *g* The supernatants were then incubated with the substances shown above for 10 min The data represent the mean ± S E of 14 replicates for the control and of six replicates for all other values

Addition	Pyruvate kinase activity [a]
None	0 082 ± 0 003
25 μM cyclic AMP	0 050 ± 0 003 [b]
75 μM cyclic GMP	0 055 ± 0 004 [b]
25 μM cyclic AMP + 75 μM cyclic GMP	0 047 ± 0 004 [b,c]
300 μM cyclic AMP	0 029 ± 0 003 [b]
500 μM cyclic GMP	0 041 ± 0 003 [b]
300 μM cyclic AMP + 500 μM cyclic GMP	0 029 ± 0 001 [b,d]

[a] Pyruvate kinase activity (nmol pyruvate generated/ml per min) is expressed as the ratio of activities with 0 3 and 4 0 mM phospho*enol*pyruvate as substrate
[b] $P < 0\ 01$ vs control
[c] $P < 0\ 05$ vs 75 μM cyclic GMP alone
[d] $P < 0\ 001$ vs 300 μM cyclic GMP alone

TABLE II

EFFECTS OF ACETYLCHOLINE OR NaN_3 ON L-FORM PYRUVATE KINASE ACTIVITY AND CYCLIC GMP CONCENTRATION IN ISOLATED RAT HEPATOCYTES

Pyruvate kinase activity is expressed as the ratio of activities, in nmol pyruvate formed/ml per min, with 0 3 and 4 0 mM phospho*enol*pyruvate as substrate Values represent the mean ± S E of 14 determinations for studies with acetylcholine and eight determinations for studies with NaN_3 GMP concentration, values represent the mean ± S E of six determinations

Addition	Pyruvate kinase activity	GMP concentration (pmol/g cell protein)
None	0 089 ± 0 005	233 ± 16
0 1 μM acetylcholine	0 091 ± 0 005	358 ± 9 [a]
1 μM acetylcholine	0 092 ± 0 005	447 ± 3 [a]
10 μM acetylcholine	0 091 ± 0 004	488 ± 21 [a]
None	0 093 ± 0 005	242 ± 14
0 1 mM NaN_3	0 091 ± 0 010	530 ± 24 [a]
0 5 mM NaN_3	0 091 ± 0 006	603 ± 17 [a]
1 0 mM NaN_3	0 092 ± 0 003	917 ± 27 [a]
5 0 mM NaN_3	0 092 ± 0 004	1323 ± 62 [a]

[a] $P < 0\ 001$ for difference between control values and other values within each group

increased GMP concentrations, but did not alter pyruvate kinase activity (Table II). Similar results (data not shown) were observed with carbamylcholine, 0.1—10 μM, or acetylcholine, 0.1—10 μM, plus eserine sulfate, 1 μM.

Discussion

The role of cyclic GMP in the regulation of hepatic glucose metabolism has not been defined clearly. Various studies have suggested that exogenous cyclic

GMP mimics the efffects of cyclic AMP on glycogenolysis [19—23] and gluconeogenesis [20,22—25]. In contrast, preliminary data suggest that hormonal stimulants of endogenous cyclic GMP accumulation may have no effect on these metabolic pathways, or may have effects opposite to those of exogenous cyclic GMP [26—28]. Discrepancies between the effects of exogenous cyclic GMP and stimulants of endogenous cyclic GMP accumulation have been noted in other systems [8,9].

The effects of cyclic GMP on glycolysis and on pyruvate kinase activity specifically have been examined in one previous study [1], but not characterized in detail. Therefore, we evaluated the effects of exogenous cyclic GMP and stimulants of endogenous cyclic GMP accumulation on hepatic pyruvate kinase activity and compared these effects with those of exogenous cyclic AMP. As reported previously [1], exogenous cyclic GMP, as cyclic AMP, decreased this enzyme activity, however, the action of cyclic GMP was less rapid and cyclic GMP was less potent than cyclic AMP. Similar results were noted with the dibutyryl forms of these cyclic nucleotides. In contrast, acetylcholine, carbamylcholine and NaN_3 had no effect on pyruvate kinase activity, whereas these agents did cause a significant increase in the levels of cyclic GMP within the hepatocytes.

The present results are similar to our previous data on cyclic GMP and renin secretion by rat kidney [9]. Those data and the present observations suggest that the actions of exogenous cyclic GMP in certain metabolic systems may not reflect the physiological effects of endogenous cyclic GMP. Our current experiments with combinations of exogenous cyclic GMP and cyclic AMP suggest that exogenous cyclic GMP and cyclic AMP may compete with each other by acting via the same mechanisms. This possibility was also raised by the results of our previous study [9]. Endogenous cyclic GMP or stimulants of its generation may have no physiologic effects on pyruvate kinase.

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