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EFFECTS OF PYRUVATE AND OTHER METABOLITES ON CYCLIC GMP LEVELS IN INCUBATIONS OF RAT HEPATOCYTES AND KIDNEY CORTEX

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Summary

Pyruvate increased cyclic GMP levels in rat hepatocytes. The effects were observed without or with 1-methyl-3-isobutylxanthine. Lactate, acetate, oxaloacetate, α-ketoglutarate, succinate, acetoacetate and β-hydroxybutyrate also increased cyclic GMP levels. Some compounds increased cyclic GMP in kidney cortex slices. The effects were dependent upon Ca^{2+} in the medium. Cyclic AMP was increased 30—50% by some of these substances with 2.6 mM Ca^{2+}. Rotenone, oligomycin, antimycin, dinitrophenol, KCN, and arsenate decreased GTP and ATP, basal cyclic GMP and the pyrubate effect, but did not alter cyclic AMP. Although fluoroacetate alone had no effect on cyclic nucleotides, GTP, or ATP, it potentiated the pyruvate effect on cyclic GMP. Adenosine and guanosine increased cyclic GMP and GTP to a similar extent of 30—50%. Aminooxyacetate, cycloserine, pentenoic acid and mepacrine decreased the pyruvate effect while cycloserine or mepacrine alone increased cyclic GMP. Citrate and mepacrine inhibited soluble and particulate guanylate cyclase from rat liver while cycloserine and acetoacetate increased guanylate cyclase activity. None of the other compounds altered guanylate cyclase activity. These results indicate that various metabolites and inhibitors can alter cyclic GMP accumulation in hepatocytes and renal cortex slices. Several mechanisms may be involved in these effects.

Abbreviation: MIX- 1-methyl-3-isobutylxanthine.

Introduction

While many hormones, autocoids, and neurotransmitters can increase cyclic GMP levels in some intact tissues, direct and specific effects of these agents on guanylate cyclase activity (GTP pyrophosphate-lyase, (cyclicing), EC 4.6.1.2) in cell-free systems have not been convincingly demonstrated [1]. A few reports have described hormone effects on guanylate cyclase activity. However, these reports have either not been confirmed or may be explained by contaminants in hormone preparations [2], nonenzymatic formation of cyclic GMP [3], and, perhaps, other nonspecific effects or interesting artifacts. Thus, current evidence suggests that hormonal regulation of guanylate cyclase is probably secondary to other processes or intervening events [1,4].

A variety of nitrocompounds and compounds such as azide or hydroxylamine that lead to the formation of nitric oxide in incubations can activate guanylate cyclase in broken cell preparations and increase cyclic GMP accumulation in intact cells [5—8]. In contrast to the effects of hormones on cyclic GMP levels [9,10], the effects of the latter compounds are not dependent upon calcium in the incubation medium [11,12]. Another reactive free radical, probably hydroxyl radical, can explain the activation of guanylate cyclase by superoxide dismutase and nitrate reductase [13]. The formation of such a reactive oxygen species and activation of guanylate cyclase may explain the effects of various hormones, drugs, and processes on cyclic GMP levels in tissues. Modulation of guanylate cyclase activity and/or cyclic GMP levels by various sulfhydryl reagents has also been described [5,8,14,15]. These and other indirect studies have indicated that guanylate cyclase activity could be regulated by intracellular redox events and subsequent oxidation and reduction of the enzyme [1,4].

Since the addition of various substrates and metabolites to tissue incubations is known to alter the redox state of cells [16], we examined the effects of pyruvate and other materials on cyclic GMP levels in isolated rat hepatocytes and renal cortex slices. Pyruvate, lactate, acetate, some tricarboxylic acid cycle intermediates, and ketone bodies increased cyclic GMP levels in these studies. While the mechanisms for these effects appear complicated and are not currently understood, clearly the metabolism of some compounds is associated with increased guanylate cyclase activity and cyclic GMP synthesis. Some of these observations have been presented in abstract form [17].

Methods

Male Sprague-Dawley rats weighing 150—250 g and fed ad libitum were used. Liver cells were prepared according to the procedure of Berry and Friend [18] with some modifications. Rats were anesthesized with pentobarbital (30 mg/kg) and the liver was perfused for 30 min at 38 ml/min with oxygenated, calcium-free Hank's solution containing 0.03—0.04% collagenase and 0.5% fatty-acid-free bovine serum albumin. The liver was removed, and incubated at 37°C for 5 min in a similar solution. The resulting cell suspension was filtered through two layers of nylon mesh and centrifuged at $30 \times g$ for 2 min. Cells were resuspended in Krebs-Ringer bicarbonate solution (pH 7.4), containing

120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and Ca^{2+} at concentrations indicated. Cell viability was checked routinely for each preparation using trypan blue exclusion and only preparations with viability in excess of 90% were used. Cell suspensions ($4 \cdot 10^6$ cells in 1.2 ml) were incubated in plastic tubes in an atmosphere of 95% O_2/5% CO_2 at 37°C with gentle shaking. Hepatocytes were pre-incubated for 15 or 20 min before the addition of various agents at the final concentrations indicated.

Kidney cortex slices were divided into two portions and preincubated in Krebs-Ringer bicarbonate solution (5 mg protein/3 ml medium) for 20 min before the addition of the agents indicated. One-half of each slice served as a control for the other half-slice that was incubated with test agent.

Incubations were terminated at the times indicated with the addition of 6% trichloroacetic acid, the tissues immediately homogenized using a Polytron, and frozen in liquid nitrogen for subsequent processing and analysis. Supernatant fractions were extracted with diethyl ether and assayed for cyclic nucleotide by radioimmunoassay methods [19] as described previously [8,12].

For GTP and ATP analyses, a 0.2 ml aliquot of homogenate was transferred to tubes with 20% perchloric acid and immersed into liquid nitrogen. Supernatant fractions were placed in ice, neutralized with K_2CO_3, and recentrifuged. Extracts were assayed for ATP content using the firefly luciferase assay [20]. GTP was assayed as ATP formed, according to the procedure of Karl [21], after nucleotide hydrolysis and treatment with transphosphorylase. Liver was also frozen between liquid nitrogen precooled clamps in intact anesthesized animals, placed in liquid nitrogen, and analyzed for GTP and ATP for in situ levels of these nucleotides.

Guanylate cyclase activity in $105\,000 \times g$ supernatant and particulate fractions of rat liver homogenates was assayed as described previously [5]. Incubations contained 50—100 μg protein/50 mM Tris-HCl buffer (pH 7.6)/10 mM theophylline/7 mM creatine phosphate/20 μg creatine phosphokinase (120—135 units/mg)/1 mM GTP/4 mM $MgCl_2$. Incubations (100 μl) were run in duplicate or triplicate for 10 min at 37°C with the agents indicated and were terminated with the addition of 0.9 ml 50 mM sodium acetate (pH 4.0) and heating at 95°C for 3 min. Cyclic GMP formed was analyzed by radioimmunoassay as described [8,12,19].

All values presented are means of triplicate or quadriplicate incubations and all experiments were performed at least three times. Values are expressed per 10^6 cells, which represents about 1.5 mg protein.

Protein was determined with the method of Lowry et al. [22] using bovine serum albumin as standard. Collagenase (Class IV, 166 units/mg) was obtained from Worthington Biochemicals Co. Other reagents were obtained as described previously [5,8,12].

Results

Effect of pyruvate on cyclic GMP levels. Pyruvate at 2—20 mM markedly increased cyclic GMP accumulation in incubations of rat hepatocytes in a concentration-dependent manner (Fig. 1). Basal concentrations of cyclic GMP were about 30—40 fmol/10^6 cells (23 fmol/mg protein) compared to values of about

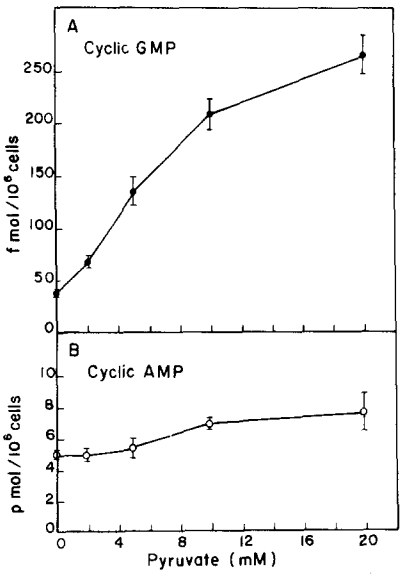

Fig. 1. Effect of pyruvate on cyclic nucleotide levels in hepatocyte incubations. Rat hepatocytes were preincubated 20 min in Krebs-Ringer bicarbonate buffer containing 2.6 mM Ca^{2+} as described. Pyruvate at the concentrations indicated was added to incubations for 5 min, and cyclic GMP (A) and cyclic AMP (B) in cells plus medium were analyzed.

5 pmol cyclic AMP/10^6 cells (3.3 pmol/mg protein). At pyruvate concentrations of less than 10 mM, cyclic AMP levels were unaltered, and with higher concentrations of pyruvate cyclic AMP increased about 50% (Fig. 1). With 10 mM pyruvate the increase in cyclic GMP over basal levels was maximal (about 4-fold) at about 5 min. At later times, the percent increase with pyruvate was similar with somewhat higher basal levels of cyclic GMP. In a number of similar experiments the maximal effect of pyruvate on cyclic GMP levels observed at 5—10 min incubation ranged from an increase of 3—8-fold over basal values.

The addition of 0.2 mM 1-methyl-3-isobutylxanthine (MIX) to incubations increased cyclic GMP levels about 2-fold and cyclic AMP levels about 30% (Table I). The effect of 5 mM pyruvate on cyclic GMP levels was enhanced with MIX while no effect of pyruvate on cyclic AMP levels was observed. The potentiation of the pyruvate effect on cyclic GMP levels with MIX suggests that pyruvate increased cyclic GMP synthesis by guanylate cyclase rather than decreased its hydrolysis by cyclic nucleotide phosphodiesterase.

Requirement for calcium for the pyruvate effect. When hepatocytes were incubated in Krebs-Ringer bicarbonate medium for 25 min without added calcium, basal cyclic GMP levels were decreased 64 and 78% compared to levels in incubations containing 1.3 mM and 2.6 mM Ca^{2+}, respectively (Table II). The presence of 1 mM EGTA in incubations caused a further small decrease in cyclic GMP. In the absence of added calcium, with or without EGTA present, 5 mM pyruvate had no effect on cyclic GMP levels. The effect of pyruvate on cyclic GMP concentrations was much greater with 2.6 mM calcium compared to 1.3 mM calcium in the incubation medium (Table II). The effect of 5 mM

TABLE I

EFFECT OF PYRUVATE AND MIX ON CYCLIC NUCLEOTIDE LEVELS IN HEPATOCYTES

Hepatocytes were incubated with Krebs-Ringer buffer containing 1.5 mM Ca^{2+} for 5 min after the addition of 5 mM pyruvate and/or 0.2 mM MIX as indicated. Values are means ±S.E.

Addition	Cyclic GMP (fmol/10^6 cells)	Cyclic AMP (pmol/10^6 cells)
None	26.1 ± 4.1	4.9 ± 0.5
Pyruvate	60.0 ± 8.1	5.2 ± 0.4
MIX	50.1 ± 4.2	6.8 ± 0.7
Pyruvate + MIX	118.2 ± 8.3	7.3 ± 0.7

pyruvate on cyclic AMP levels was also dependent upon calcium concentrations in the incubation medium. With no calcium or 1.3 mM calcium, 5 mM pyruvate had no effect on cyclic AMP, whereas with 2.6 mM calcium cyclic AMP was increased about 30%. In contrast to the effects of calcium and EGTA on basal cyclic GMP levels they did not alter basal cyclic AMP levels.

A variety of other metabolites also increased cyclic GMP levels in hepatocyte incubations (Table III). Lactate at 10 mM concentrations increased cyclic GMP levels 3.0-fold. Acetate, some intermediates of the tricarboxylic acid cycle, such as oxaloacetate, α-keto-glutarate and succinate, as well as acetoacetate and β-hydroxybutyrate also increased cyclic GMP levels. Citrate decreased cyclic GMP levels while fumarate and glucose had no effect. The effects of these agents were concentration-dependent in that compounds tested at 5 mM had less of an effect or no effect. The effects of these other substances on cyclic GMP levels were also dependent upon the addition of calcium to the incubation medium. The agents listed in Table III that increased cyclic GMP levels with 1.5 mM Ca^{2+} in the incubation media had no effect in the absence of added calcium. Whereas none of these agents altered GTP or ATP levels in the absence or

TABLE II

CALCIUM DEPENDENCY OF THE PYRUVATE EFFECT ON CYCLIC NUCLEOTIDES IN HEPATOCYTES

Hepatocytes were incubated for 25 min in Krebs-Ringer buffer containing no Ca^{2+}, 1.3 mM Ca^{2+}, 2.6 mM Ca^{2+}, or 1 mM EGTA as indicated. Some incubations contained 5 mM pyruvate for the last 5 min of incubation. Values are means ±S.E.

Addition	Concn. (mM)	Cyclic GMP (fmol/10^6 cells)	Cyclic AMP (pmol/10^6 cells)
No Ca^{2+}			
None		24.1 ± 4.2	6.3 ± 0.3
Pyruvate	5	24.5 ± 4.3	6.6 ± 0.4
EGTA	1	22.1 ± 4.0	6.0 ± 0.3
EGTA + Pyruvate		24.7 ± 2.8	6.1 ± 0.3
1.3 mM Ca^{2+}			
None		40.3 ± 4.7	6.6 ± 0.5
Pyruvate	5	103.8 ± 8.7	6.7 ± 0.4
2.6 mM Ca^{2+}			
None		108.4 ± 18.3	6.4 ± 0.8
Pyruvate	5	369.7 ± 47.9	9.1 ± 0.8

TABLE III

EFFECTS OF PYRUVATE AND OTHER METABOLITES ON CYCLIC GMP LEVELS IN RAT HEPATOCYTE INCUBATIONS

Hepatocytes were incubated in Krebs-Ringer bicarbonate buffer containing 1.5 mM Ca^{2+}. Reactions were stopped 5 min after the addition of the agents indicated. Values are means ±S.E. of triplicate incubations.

Addition	mM	Cyclic GMP	
		fmol/10^6 cells	Percentage of control
Experiment 1			
None		23.2 ± 1.1	100
Pyruvate	10	101.2 ± 12.3	436
Lactate	10	68.8 ± 6.5	297
Citrate	10	13.5 ± 0.7	58
α-Ketoglutarate	10	50.3 ± 4.7	217
Succinate	10	96.0 ± 10.5	414
Fumarate	10	25.5 ± 4.2	110
Oxaloacetate	10	39.4 ± 5.3	170
Experiment 2			
None		33.5 ± 2.8	100
Acetate	10	147.4 ± 15.6	440
Acetoacetate	10	115.9 ± 10.7	346
β-Hydroxybutyrate	10	105.5 ± 7.3	315
Experiment 3			
None		21.0 ± 1.9	100
Glucose	10	24.5 ± 2.3	117
Pyruvate	5	68.0 ± 4.1	324
Glucose + Pyruvate	10,5	67.1 ± 5.2	320

presence of added calcium, with 2.6 mM Ca^{2+} 10 mM acetate, citrate, succinate, or acetoacetate also increased cyclic AMP levels and the effects were similar to those of pyruvate and Ca^{2+}.

Pyruvate also increased cyclic GMP levels in kidney cortex incubations but the effect was much less than that observed with hepatocytes (Table IV). At a similar concentration of 10 mM, acetate, α-ketoglutarate, and succinate increased cyclic GMP to a lesser degree.

Effects of metabolites and calcium on GTP and ATP levels. Although the omission of calcium from the incubation medium decreased cyclic GMP levels

TABLE IV

EFFECTS OF PYRUVATE AND OTHER METABOLITES ON CYCLIC GMP LEVELS IN RAT KIDNEY CORTEX SLICES

Rat kidney cortex slices were preincubated 20 min before the addition of the agents indicated at 10 mM for 5 min. Values are means ±S.E. of four incubations. The percentage of basal values were determined using one-half of the slice as control and the other half of the slice for the test agent.

Addition	Cyclic GMP (fmol/mg protein)		
	Basal	+Addition	Percentage of basal
Pyruvate	234.9 ± 28.9	421.4 ± 33.0	179
Lactate	227.7 ± 18.7	246.1 ± 11.6	108
Acetate	168.7 ± 31.3	216.6 ± 20.9	128
α-Ketoglutarate	213.8 ± 26.7	243.8 ± 16.8	114
Succinate	282.8 ± 13.4	387.3 ± 13.7	137

in hepatocytes, the concentrations of GTP and ATP as well as cyclic AMP were unaltered by the presence or absence of calcium. The effects of pyruvate and the other compounds tested on cyclic nucleotide levels also occurred without alterations in the levels of GTP or ATP. GTP and ATP levels were 4.6 and 36 nmol/10^6 cells, respectively, with or without the addition of calcium or other agents that altered cyclic GMP or cyclic AMP levels.

Effects of metabolic inhibitors. Rotenone, oligomycin, antimycin, dinitrophenol, cyanide, and arsenate decreased basal cyclic GMP levels as well as concentrations of GTP and ATP (Table V). In contrast, these agents had little or no effect on cyclic AMP levels in hepatocyte incubations. The effect of pyruvate on cyclic GMP levels was also decreased by these compounds. Fluoroacetate at 1 or 2 mM increased the effect of pyruvate on cyclic GMP levels and had no effect on cyclic AMP, GTP or ATP. These studies indicate that cyclic AMP levels in hepatocytes are not sensitive to alterations in GTP and ATP levels. The effect of pyruvate to increase cyclic GMP levels could not be explained by alterations in GTP or ATP concentrations. However, alterations in GTP levels with some compounds were associated with significant changes in cyclic GMP (Table V). The addition of 0.25 mM adenosine or guanosine to incubations increased GTP and cyclic GMP levels to a similar degree of about 30—50%. These studies suggest that mechanisms other than the availability of GTP or ATP were involved in the effect of pyruvate to increase cyclic GMP levels.

Aminooxyacetate, an inhibitor of the malate-aspartate shuttle and fatty acid synthesis [23], decreased the effect of pyruvate on cyclic GMP levels without altering the concentrations of GTP or ATP (Table VI). However, cycloserine, an inhibitor of the same category, increased cyclic GMP levels when added alone at 5 mM. Cycloserine had no effect on GTP or ATP levels and its effect on cyclic GMP levels may be attributable to the stimulatory effect of this compound on guanylate cyclase activity (see below). Pentenoic acid, an inhibitor of fatty acid oxidation [24—26], also decreased the effect of pyruvate. Mepacrine, an inhibitor of oxidative phosphorylation and phospholipase A_2 [27,28], had a small stimulatory effect on basal cyclic GMP levels, and decreased levels of GTP and ATP (Table VI). Mepacrine also decreased the effect of pyruvate on cyclic GMP accumulation.

Effect of some compounds on guanylate cyclase activity. The activities of guanylate cyclase in soluble and particulate fractions of rat liver homogenates assayed with 4 mM Mg^{2+} were 4.62 ± 0.34 and 0.39 ± 0.03 pmol/mg protein per min, respectively, (Table VII). Citrate at 5 or 10 mM markedly decreased soluble and particulate guanylate cyclase activities. Mepacrine at 1 mM was slightly inhibitory, while cycloserine at 5 mM increased soluble and particulate guanylate cyclase activity 20 and 80%, respectively. The stimulatory effect of acetoacetate with soluble but not particulate guanylate cyclase was about 2-fold with 20 mM acetoacetate in other experiments. None of the other compounds tested altered soluble or particulate guanylate cyclase activity. The other compounds tested included 10 mM pyruvate, lactate, α-ketoglutarate, succinate, fumarate, oxalacetate, acetate, β-hydroxybutyrate or pentenoate. 2 μM rotenone, 1 μg/ml oligomycin, 5 μg/ml antimycin, 0.1 mM dinitrophenol, 2 mM KCN, 0.1 mM arsenate, 2 mM fluoroacetate or 5 mM aminooxyacetate also had no effect on soluble or particulate guanylate cyclase activity.

TABLE V

EFFECTS OF PYRUVATE AND VARIOUS INHIBITORS ON GTP, ATP AND CYCLIC NUCLEOTIDE LEVELS IN RAT HEPATOCYTES

Hepatocytes were incubated in Krebs-Ringer bicarbonate buffer containing 1.5 mM Ca^{2+}. Inhibitors were added 5 min prior to the addition of 5 mM pyruvate which was present for 5 min as indicated. Values are presented as percent of basal values. Basal values (no pyruvate or inhibitor) of each nucleotide were: cyclic GMP, 31.7 ± 2.4 fmol/10^6 cells; GTP, 4.28 ± 0.44 nmol/10^6 cells; cyclic AMP, 5.67 ± 0.31 pmol/10^6 cells; ATP, 30.3 ± 2.3 nmol/10^6 cells.

Addition	Concentration	Cyclic GMP		GTP		Cyclic AMP		ATP	
		—Pyruvate	+Pyruvate	—Pyruvate	+Pyruvate	—Pyruvate	+Pyruvate	—Pyruvate	+Pyruvate
Experiment 1									
None		100	211	100	102	100	107	100	101
Rotenone	1 μM	101	221	76	83	92	92	71	79
	2 μM	93	169	63	69	95	106	63	57
Oligomycin	1 μg/ml	83	143	36	40	103	113	28	30
Antimycin	5 μg/ml	23	34	4	9	82	91	4	8
Dinitrophenol	50 μM	83	209	82	85	92	123	69	70
	100 μM	50	104	38	61	94	102	30	43
KCN	2 mM	91	179	90	94	98	101	74	76
Arsenate	0.1 mM	63	111	74	72	90	89	72	72
Experiment 2									
None		100	203	100	106	100	110	100	106
Fluoroacetate	1 mM	105	304	96	103	99	115	96	104
	2 mM	104	402	97	99	97	118	95	98

TABLE VI

EFFECTS OF PYRUVATE AND VARIOUS INHIBITORS ON GTP, ATP AND CYCLIC NUCLEOTIDE LEVELS IN HEPATOCYTE INCUBATIONS

Hepatocytes were incubated as described in the legend to Table V. Values presented are percent of basal levels.

Addition	Concentration (mM)	Cyclic GMP		GTP		Cyclic AMP		ATP	
		—Pyruvate	+Pyruvate	—Pyruvate	+Pyruvate	—Pyruvate	+Pyruvate	—Pyruvate	+Pyruvate
None		100	234	100	105	100	106	100	103
Aminooxyacetate	1	92	143	95	98	99	109	94	95
	5	96	106	99	96	116	112	101	102
Cycloserine	5	427	378	101	102	98	107	101	99
Pentenoate	10	94	166	102	96	94	96	97	97
Mepacrine	1	147	128	90	96	102	102	72	79

TABLE VII

EFFECTS OF SOME COMPOUNDS ON SOLUBLE AND PARTICULATE GUANYLATE CYCLASE FROM RAT LIVER

Guanylate cyclase was assayed in the presence of the agents indicated.

Addition	Concn. (mM)	Cyclic GMP formed (pmol/mg protein per min)	
		Soluble	Particulate
None		4.62	0.39
Citrate	10	1.33	0.06
Acetoacetate	10	5.35	0.41
Cycloserine	5	5.54	0.70
	10	6.80	1.13
Mepacrine	1	3.64	0.26

Discussion

Pyruvate, lactate, acetate, various tricarboxylic acid cycle intermediates, and several ketone bodies increased cyclic GMP accumulation in incubations of rat hepatocytes. Some of these compounds also increased cyclic GMP levels in incubations of rat renal cortex slices. However, the increases in cyclic GMP were much less with kidney preparations. The effect of pyruvate was concentration-dependent and was rapid with maximal increases in cyclic GMP levels within 5 min. The enhancement of the pyruvate effect with MIX suggests that pyruvate increases cyclic GMP synthesis rather than decreases the rate of hydrolysis by cyclic nucleotide phosphodiesterase. Similar to the effects of various hormones and neurotransmitters on cyclic GMP accumulation in several tissues [9,10,12], the effect of pyruvate was calcium-dependent. The omission of calcium from the incubation medium decreased basal levels of cyclic GMP and the effect of pyruvate was absent. Removing calcium from incubations of hepatocytes did not alter basal levels of cyclic AMP, ATP, or GTP. Schultz et al. [9] reported that GTP levels in rat ductus deferens were not altered with the removal of calcium. However, Pointer et al. [29] found decreased ATP levels in hepatocytes using calcium-free medium. The reason for the difference in the effects of calcium on ATP levels in the latter study and the work reported here is unknown.

It seems quite unlikely that the effects of pyruvate on cyclic GMP levels are due to altered levels of GTP as substrate for guanylate cyclase or to changes in ATP levels which can inhibit soluble and particulate guanylate cyclase [30]. However, these possibilities cannot be excluded entirely at present. Pyruvate and other metabolites that increases cyclic GMP levels did not alter basal levels of GTP or ATP. Basal levels of ATP were 2.5 and 2.74 μmol/g wet weight in hepatocytes and intact liver, respectively. These values are similar to earlier reports [31,32]. Concentrations of GTP were 0.38 and 0.41 μmol/g wet weight in hepatocytes and intact liver, respectively. The similarity of nucleoside triphosphate levels in hepatocytes and intact liver is consistent with the hepatocyte viability of 90—95%, as determined using trypan blue exclusion routinely with each preparation.

Inhibitors and uncouplers of electron transport decreased basal levels of cyclic GMP, GTP, and ATP, and decreased the effect of pyruvate on cyclic GMP accumulation, whereas basal levels of cyclic AMP were decreased very little, if any. When GTP or ATP levels were increased 30—50% with adenosine or guanosine, comparable increases in cyclic GMP levels were observed. Considering the GTP concentrations in hepatocytes (about 0.4 mM) and the apparent K_m for GTP of soluble and particulate guanylate cyclase of about 10 to 100 μM [1], the availability of GTP as substrate for guanylate cyclase may be rate-limiting under some conditions. On the other hand, marked alterations in ATP levels in hepatocytes were not accompanied by changes in cyclic AMP levels. The effects of pyruvate and some other compounds on cyclic GMP accumulation are probably not due to altered levels of GTP or ATP, as determined in extracts of hepatocyte incubations. Some compounds such as aminooxyacetate, pentenoic acid and mepacrine decreased the effect of pyruvate on cyclic GMP accumulation without altering GTP levels. Furthermore, fluoroacetate increased the effect of pyruvate on cyclic GMP, had no effect alone on cyclic GMP, and did not alter ATP or GTP. These studies suggest that pyruvate and probably other metabolites increased guanylate cyclase activity and cyclic GMP levels through mechanisms other than altering GTP or ATP levels. However, we cannot exclude changes in nucleoside triphosphate levels in some compartments that are not reflected in measurements of the entire hepatocyte extract.

At present we cannot explain the mechanism of the pyruvate effect on cyclic GMP levels in hepatocytes and renal cortex. The requirement for calcium, the potentiation of the pyruvate effect with fluoroacetate, and the inhibition of the pyruvate effect with a fatty acid synthesis inhibitor (aminooxyacetate), a fatty acid oxidation inhibitor (pentenoid acid), mepacrine and electron transport inhibitors suggest that several mechanisms may be involved. What has become quite apparent from these studies is that some metabolic events involving pyruvate, acetate and other compounds are coupled to guanylate cyclase activation and cyclic GMP synthesis. Cycloserine activated soluble and particulate guanylate cyclase from liver while citrate and mepacrine were inhibitory. Whereas citrate inhibition of guanylate cyclase may be related to its metal chelating properties, this was not examined. Krause et al. [33] have also observed citrate inhibition of soluble guanylate cyclase from heart.

Since guanylate cyclase can be activated by free radicals such as nitric oxide [8] and probably hydroxyl radical [13] and cyclic GMP levels in some tissues can be influenced by oxidizing and/or reducing agents [34,35], it may be that the metabolism of pyruvate and some other materials results in alterations in guanylate cyclase activity through some calcium-dependent redox mechanism. While this hypothesis may explain the effects of pyruvate, some hormones, and drugs on cyclic GMP metabolism, additional investigation is needed since lactate and other compounds have similar effects.

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