EFFECTS OF MANGANESE ON THE ACTIVITY OF GLYCOLYTIC AND GLUCONEOGENIC ENZYMES IN THE PERFUSED RAT LIVER

Janet M. WIMHURST and K.L. MANCHESTER*

Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, England

Received 17 October 1972

1. Introduction

We have shown previously [1] that pyruvate carboxylase activity declines when liver from the fasted rat is perfused with glucose containing medium. Conversely addition of lactate to the perfusate enhances the activity of both pyruvate carboxylase and phosphoenolpyruvate carboxykinase [2]. It appears that the hepatic activity of these two enzymes may fluctuate depending on the concentration of the final product and on the supply of precursors for transformation. Administration of manganese results in a rapid increase in serum glucose [3] and is reported to enhance glucose production by the perfused liver [4]. It is a potent activator of several glycolytic and gluconeogenic enzymes including specifically pyruvate carboxylase and phosphoenolpyruvate carboxykinase [5, 6]. We here report that addition of manganese to the perfused rat liver prevents the increase in activity of the two enzymes otherwise induced by lactate. This seemingly contradictory event is interpreted to mean that the extra functional capacity of pyruvate carboxylase and phosphoenolpyruvate carboxykinase resulting from manganese activation minimises the influences which would otherwise induce extra enzyme synthesis.

2. Methods

Livers from 150 g male rats fasted overnight were perfused for 4 hr as previously described [1] with minor modifications. The perfusate consisted of 40% fresh blood taken from fasting rat donors and was diluted with Krebs Ringer bicarbonate and heparin (10,000 units) to a final volume of 80 ml, haematocrit 20% and flow rate 6 ml per min. Lactate was added initially at 18 mM and the perfusate concentration maintained by infusion of 10.3 μmoles per min throughout the period of the experiment. $MnCl_2$ when added was 50 μM in both perfusate and infusion. Mn content of the liver was measured by atomic absorption spectrophotometry.

Gluconeogenic, glycolytic and other enzymes were assayed as described previously [6, 7]. Perfusate glucose, lactate, pyruvate, urea and amino acids, liver K^+, DNA, RNA, glycogen, amino acids and water content were also measured [1].

3. Results

Table 1 compares the activities of the uniquely glycolytic and gluconeogenic enzymes in livers perfused for 4 hr with 18 mM lactate in the presence and absence of manganese with the activities of the enzymes in control livers perfused briefly with 10 ml ice cold saline to remove blood.

Activity of both pyruvate carboxylase and phosphoenolpyruvate carboxykinase was significantly higher in livers perfused with lactate than in the control livers. This increase did not occur when cycloheximide was present [2]. Addition of $MnCl_2$ to the perfusate prevented increase in the activity of pyruvate carboxylase, whilst activity of both phosphophenolpyruvate carboxykinase and fructose 1,6-diphosphatase were significantly lower relative to controls and to lactate

* Present address: Department of Biochemistry, University of the West Indies, Mona, Kingston 7, Jamaica.

Table 1
Effect of addition of manganese chloride on the activities of glycolytic and gluconeogenic enzymes in livers perfused with high concentrations of lactate.

	Activities (μmole/g/min)		
	Before perfusion	Perfusion with lactate alone	Manganese chloride added
Glucokinase	3.51 ± 0.29 (8)	3.10 ± 0.32 (4)	3.13 ± 0.18 (4)
Hexokinase	0.49 ± 0.02	0.48 ± 0.02	0.49 ± 0.03
Phosphofructokinase	7.15 ± 0.28	6.65 ± 0.41	6.38 ± 0.46
Pyruvate kinase	74 ± 4.6	73 ± 9.1	72 ± 5.8
Pyruvate carboxylase	6.12 ± 0.18	9.43 ± 0.45***	6.16 ± 0.23†††
Phosphoenolpyruvate carboxykinase	9.7 ± 0.22	11.4 ± 0.56**	7.8 ± 0.73*††
Fructose 1,6-diphosphatase	12.2 ± 0.55	10.6 ± 0.64	8.3 ± 0.54**†
Glucose 6-phosphatase	19.3 ± 1.2	15.6 ± 0.71	16.6 ± 1.4

Each figure is the mean ± S.E.M. of the number in parentheses. *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$ for difference with respect to zero time. †$p < 0.05$, ††$p < 0.01$, †††$p < 0.001$ for difference due to manganese. Lactate (18 mM) was added to the perfusate initially followed by a constant infusion of 10.3 μmole/min to maintain the lactate concentration at approx. 18 mM. Manganese chloride when added was 50 μM.

Table 2
Non-enzymic parameters of livers perfused with lactate and manganese chloride.

	Before perfusion	Perfusion with lactate alone	Manganese chloride added
DNA (μg DNA-P_i/g)	353 ± 9 (8)	344 ± 4 (4)	358 ± 13 (4)
RNA (μg RNA-P_i/g)	961 ± 32	905 ± 22	883 ± 28
RNA/DNA	2.72 ± 0.06	2.62 ± 0.06	2.47 ± 0.05*
Protein (mg/g)	253 ± 7	236 ± 5	222 ± 1.5*†
Glycogen (mg/g)	0.26 ± 0.09	1.73 ± 0.54**	1.07 ± 0.38
Lipid (mg/g)	30.2 ± 1.4	26.3 ± 3.4	31.5 ± 4.4
Water/solid	2.81 ± 0.09	3.05 ± 0.03	2.97 ± 0.05
Potassium (μmole/g)	94 ± 3.4	93 ± 6.4	94 ± 4.8
Manganese (nmole/g)	53 ± 6.5	52 ± 4.3	352 ± 34°

Each figure is the mean ± S.E.M. of the number in parentheses. *$p < 0.05$, **$p < 0.01$, for difference with respect to zero time. †$p < 0.05$ for difference due to manganese. °$p < 0.001$ with respect to both controls. Livers described in the table are the same as those used for enzyme assays of table 1.

perfusions. No significant changes in activity were seen for any of the other enzymes assayed.

Comparisons of the ratios of the total assayable activities of the enzymes at the three possible futile cycles of the glycolytic/gluconeogenic pathways show that in the lactate perfusions the ratios change as would be expected to facilitate glucose synthesis. Pyruvate kinase versus pyruvate carboxylase and versus phosphoenolpyruvate carboxykinase ratios fall from 12 to 7.9 and from 7.6 to 6.5, respectively, relative to the controls. In the presence of $MnCl_2$ pyruvate kinase versus carboxylase is unchanged. Furthermore, pyruvate kinase versus phosphoenolpyruvate carboxykinase at 9.4 and phosphofructokinase versus fructose 1,6-diphosphatase ratios are both greater than in lactate perfusions or controls.

Although Friedmann and Rasmussen [4] showed clear stimulation of glucose production in 1 hr when manganese was added to the perfusate, we were unable to show a similar rise. Rates of glucose efflux in the first hour of 0.73 ± 0.07 and 0.65 ± 0.15 μmole per g per min for lactate and lactate plus manganese perfusions, respectively, were very similar to the manganese stimulated data of Friedmann and Rasmussen.

Estimation of blood glycolysis in the two types of perfusion gave corrected rates of glucose production of 0.92 ± 0.05 and 0.98 ± 0.16 μmole per g per min for the first hour for lactate and manganese containing perfusions, in good agreement with other published data [8, 9]. Allowing for glycogen deposition, the overall rates for the 4 hr period were 0.69 ± 0.07 and 0.80 ± 0.13 μmole per g per min for lactate and lactate plus manganese, respectively.

Hepatic concentrations of DNA and RNA showed no difference in the three groups, but there were small decreases in both the RNA/DNA ratios and in protein concentrations in the manganese perfusions (table 2). No changes were found in hepatic lipid, water, amino acid and potassium concentrations. The capacity of the perfused liver to concentrate manganese is demonstrated by the 7-fold increase in the amount of this cation in livers perfused with $MnCl_2$. All perfusions showed significant deposition of glycogen. Pyruvate and amino acid in the perfusate rose steadily to give increases of 8.9 ± 1.2 and 10.5 ± 2.3 μmoles per g for pyruvate and 0.41 ± 0.04 and 0.40 ± 0.07 mg amino acid nitrogen per g in 4 hr in lactate and lactate plus manganese experiments. Urea synthesis, 0.84 ± 0.07 and 0.76 ± 0.11 mg urea nitrogen produced per g in 4 hr, also was not significantly changed.

4. Discussion

Control of a metabolic pathway may be effected either by change in the quantity of key enzymes or by control of their activity. Demand for gluconeogenesis fasting conditions, availability of glucose precursors or hormonal stimulation seems to result in increased synthesis of enzyme protein as judged by prevention of the changes with inhibitors [2, 10]. The present results appear to offer an example of the second form of activation, namely that provision of an activating ion may minimise the need for new enzyme synthesis since the existing molecules will now work more effectively.

Activation of enzymes by manganese is often optimal at substantially lower concentrations of the ion than is the case with magnesium. It is likely that the intracellular concentration of free magnesium is comparatively low and maybe below optimal values. This will be a circumstance in which small amounts of manganese may have a potent effect. Manganese is absorbed rapidly on administration [11] and as indicated in table 2 concentrated from the perfusate, though of course it is not known where in the cell the ion resides.

In the experiments of Friedmann and Rasmussen [4] addition of manganese increased the hepatic content of malate. This could arise either directly from increased carboxylation of pyruvate or as a result indirectly of increased citrate synthesis and its efflux from the mitochondria [12]. In either case it suggests that activation of pyruvate carboxylase is a greater stimulus to gluconeogenesis than of phosphoenolpyruvate carboxykinase despite the great sensitivity of the latter enzyme to the ion [5, 6].

Acknowledgement

We are grateful to the Medical Research Council for support for this work.

References

[1] J.M. Wimhurst and K.L. Manchester, FEBS Letters 8 (1970) 91.
[2] J.M. Wimhurst and K.L. Manchester, in preparation.
[3] P.S. Papavasiliou, S.T. Miller and G.C. Cotzias, Nature 220 (1968) 74.
[4] N. Friedmann and H. Rasmussen, Biochim. Biophys. Acta 222 (1970) 41.
[5] D.O. Foster, H.A. Lardy, P.D. Ray and J.B. Johnston, Biochemistry 6 (1967) 2120.
[6] J.M. Wimhurst and K.L. Manchester, FEBS Letters 10 (1970) 33.
[7] J.M. Wimhurst and K.L. Manchester, FEBS Letters 27 (1972) 321.
[8] R. Hems, B.D. Ross, M.N. Berry and H.A. Krebs, Biochem. J. 101 (1966) 284.
[9] J.H. Exton and C.R. Park, J. Biol. Chem. 242 (1967) 2622.
[10] G. Weber, R.L. Singhal, N.B. Stamm and S.K. Srivastava, Federation Proc. 24 (1965) 745.
[11] A.J. Bertinchamps, S.T. Miller, J.C. Cotzias, Am. J. Physiol. 211 (1966) 217.
[12] E.J. Harris, C. Berent and J. Wimhurst, FEBS Letters 6 (1970) 93.