THE EFFECT OF AEROBIC PREINCUBATION ON ANAEROBIC GLYCOGENOLYSIS IN LIVER SLICES

G.GAJA and M.Elena FERRERO
Institute of General Pathology, University of Milan, 20133 Milan, Italy

Received 16 December 1969

1. Introduction

A short preliminary exposure of liver slices to oxygen causes an increase in the rate of glycolysis during subsequent anaerobiosis [1]. In previous papers we described various aspects of this stimulation [2–4]; in particular, from studies on the concentration-pattern of some phosphorylated glycolytic intermediates during "basal" and "stimulated" anaerobic glycolysis, we suggested that the limiting step mainly affected by the preliminary exposure to oxygen might be at the level of the utilization of triose phosphates [4]. Though the utilization of triose phosphates is faster in preincubated than in non-preincubated slices, stimulated anaerobic glycolysis is accompanied by an increase in the concentration of hexose monophosphates, which by contrast decreases under basal conditions. The rise in concentration of hexose monophosphates cannot be due to a decline of phosphofructokinase activity, because it occurs during an anaerobic incubation - where the activity of phosphofructokinase tends to increase [5] - and is accompanied by an increased production of lactate. Therefore, we have put forward the hypothesis that an increase in glycogenolysis might be responsible for this change. To test this hypothesis we have now studied glycogen breakdown, glucose production and lactate production in liver slices both from fed rats and from starved, glucose-injected rats: in this second group of animals, the anaerobic glycolytic rate is in the range of starved rats, but in contrast to starved rats preincubation in oxygen brings about a remarkable increase in lactate production.

Owing to their low glycogen content the livers of starved rats are not a suitable material for the study of glycogenolysis: in starved rats stimulation of glycolysis does not occur, the levels of glucose 6-phosphate are very low and further decrease during the anaerobic incubation, both in preincubated and non-preincubated liver slices.

2. Methods

Experimental conditions were those previously described [4]. The anaerobic incubation was carried out at 37°C under $N_2 + CO_2$ (95:5). When appropriate, preincubation in $O_2 + CO_2$ (95:5) was carried out at 37°C for 10 min: the flasks were then thoroughly re-gassed with $N_2 + CO_2$ (95:5) and the experiment was started 3 min after the end of the preincubation. Each flask contained about 15 slices in 5 ml of medium. Perchloric acid (final conc. 3%) was added to the flasks at 0 time and after 30 min of anaerobic incubation: the contents were then transferred to a Potter tube and homogenized. An aliquot of the homogenate was taken immediately for the determination of protein [6]: the remainder, after appropriate extraction, was utilized for the determination of glycogen [7], free glucose [8] and lactate [9].

3. Results

We will briefly comment on only those results (table 1) that are relevant to the problem under investigation.

During the anaerobic incubation, in the absence of substrate, the glycogen which disappeared from liver

Table 1

Effect of oxygen preincubation on anaerobic glycogenolysis in rat liver slices. Changes in the concentrations of glycogen, glucose and lactate in the system (slices + medium) during 30 minutes of anaerobic incubation of non-preincubated and oxygen-preincubated rat liver slices. Glucose and fructose, when present in the medium were at a concentration of 30 mM. Results are expressed as μmoles of glucose equivalents/100 mg. of protein, and are given as means ± s.e.m. of 4 experiments. Glycogen content at 0 time was about 140 and 40 μmoles of glucose equivalents/100 mg. of protein, in liver slices from fed and glucose-injected starved rats, respectively. Significance ($P < 0.05$) was assessed by the t test for paired variates, considering that, within each experiment, the slices were obtained from the same liver.

	Substrate	Glycogen		Glucose		Lactic acid	
		non preincubated	preincubated	non preincubated	preincubated	non preincubated	preincubated
Fed rats	no substrate	−31.7 ± 2.92	−48.5 ± 3.24*	+24.7 ± 1.57	+30.0 ± 1.52*	+ 8.8 ± 1.08	+16.0 ± 0.58*
	glucose	−20.4 ± 0.82†	−38.6 ± 2.79*	–	–	+ 6.2 ± 0.69	+17.4 ± 0.55*
	fructose	−44.5 ± 1.47†	−40.9 ± 3.38	+36.8 ± 0.84	+33.6 ± 1.56	+22.9 ± 1.17	+24.8 ± 1.44
Starved glucose-injected rates	no substrate	−16.6 ± 3.26	−26.8 ± 6.07*	+ 7.1 ± 0.95	+16.2 ± 3.60*	+ 1.1 ± 0.33	+ 8.9 ± 1.37*
	glucose	−10.9 ± 2.50†	−25.1 ± 5.25*	–	–	+ 1.3 ± 0.47	+11.4 ± 0.94*
	fructose	−24.3 ± 4.57†	−24.1 ± 5.28	+16.6 ± 4.41	+17.9 ± 4.42	+10.3 ± 1.84	+17.4 ± 1.97*

* significant vs. non-preincubated.
† significant vs. no-substrate.

slices of fed animals is satisfactorily accounted for by glucose and lactate recovered in the system (slices plus medium). Previous incubation in oxygen activates anaerobic glycogenolysis and causes an increase both in glucose and lactate production. Glucose has a "sparing effect" on glycogen, but in its presence glycogenolysis is activated all the same by prior exposure to oxygen, with a concurrent increase in lactate production: changes in glucose production, in the presence of such a high concentratiom of glucose in the medium cannot be followed with any accuracy. Fructose increases glycogen breakdown to a point which is not further affected by previous incubation in oxygen. The sum of glucose plus lactate recovered in the system exceeds the loss of glycogen, indicating that some fructose is either directly transformed into lactate or, less likely, converted to glucose. As previously demonstrated [2] glycolysis by liver slices from fed rats is very high in the presence of fructose but cannot be further stimulated by preliminary incubation in oxygen.

In starved, glucose-injected rats, which have a moderate glycogen content at the start of the experiment, the absolute amount of glycogen that disappeared during the anaerobic incubation is less than in fed rats: also in this group of rats, both in the absence of added substrate and in the presence of glucose, previous incubation in oxygen appreciably increases glycogen breakdown and lactate production; glycogenolysis in twice as fast, glycolysis about 8 times greater.

Fructose has a stimulatory effect on glycogen breakdown, which is not further activated by the aerobic preincubation where as lactate production, which is higher in the presence than in the absence of fructose, is further stimulated by preincubation in oxygen: most of the extra lactate is likely to be formed from the added substrate.

From this point of view starved glucose-injected rats behave as starved rats, where preincubation in oxygen causes a stimulation of lactate production in the presence of fructose.

4. Discussion

The meaning of these results can be best understood in the context of the changes in glucose 6-phosphate concentration occurring during incubation [4]. The increase in glycogen breakdown in preincubated liver slices from fed rats has its counterpart in the increase in the concentration of glucose 6-phosphate

during stimulated glycolysis. In the presence of fructose, the concentration of glucose 6-phosphate is kept uniformly high, both in preincubated and non-preincubated slices, and accordingly glycogenolysis is high under both conditions. In fed rats fructose mimics the effect of aerobic preincubation on glycogen breakdown: preincubation cannot further stimulate glycogenolysis, which is already fully released. These results support the idea that in our previous experiments the accumulation of glucose 6-phosphate in preincubated, anaerobically glycolysing liver slices is mainly sustained by an increased glycogen breakdown.

In starved, glucose-injected animals the relationships between changes in glucose 6-phosphate concentration and rate of glycogenolysis are not so clear-cut, but on the whole we think that they fit in the frame of our hypothesis, for the following reasons: i) the absolute amounts of glycogen which are broken down are considerably less than in fed animals, and reflect the different liver glycogen levels of the two groups of animals. The fact that the levels of glucose 6-phosphate mirror the amounts of glycogen in the tissue has already been shown by Young [10]. ii) The increase of glycogenolysis is associated with a much greater stimulation of lactate production. Therefore the increased speed of the glycolytic steps after hexose phosphates, which is greater than the acceleration of glycogenolysis, can explain why glucose 6-phosphate does not accumulate in preincubated liver slices from glucose-injected starved rats. In any case, glucose 6-phosphate in stimulated glycolysis stays at a steady level, while under basal conditions it tends to decrease progressively.

The increased anaerobic glycogenolysis caused by O_2-preincubation, as well as by the presence of fructose in the medium, which both increase lactate formation, underlines the existence of a still poorly defined, reciprocal relationship between glycogen breakdown and glycolytic rate in the liver.

Acknowledgements

We wish to thank Professor A.Bernelli-Zazzera for helpful discussion and criticism.

This work was supported by a grant from the Consiglio Nazionale delle Ricerche, Rome, Italy.

References

[1] O.Rosenthal and A.Lasnitzki, Biochem. Z. 196 (1928) 340.
[2] A.Bernelli-Zazzera, G.Gaja and G.Ragnotti, Biochem. J. 100 (1966) 114.
[3] G.Gaja, G.Ragnotti, F.Cajone and A.Bernelli-Zazzera, Biochem. J. 105 (1967) 647.
[4] G.Gaja, G.Ragnotti, F.Cajone and A.Bernelli-Zazzera, Biochem. J. 109 (1968) 867.
[5] A.H.Underwood and E.A.Newsholme, Biochem. J. 95 (1965) 868.
[6] E.Layne, Methods in Enzymology, vol. 3, eds. S.P.Colowick and N.O.Kaplan (Academic Press Inc., New York, 1957) p. 450.
[7] H.A.Krebs, D.A.H.Bennet, P.de Gasquet, T.Gascoyne and T.Yoshida, Biochem. J. 86 (1963) 22.
[8] H.A.Krebs, C,Dierks and T.Gascoyne, Biochem. J. 93 (1964) 112.
[9] H.J.Hohorst, Methods of Enzymatic Analysis, ed. H.U. Bergmeyer (Academic Press Inc., New York, 1963) p. 266.
[10] D.A.Young, Archs Biochem. Biophys. 114 (1966) 309.