EFFECT OF GLUCOSE FEEDING TO FASTED RATS ON THE TRANSLATIONAL EFFICIENCY OF LIVER CYTOSOL PHOSPHOENOLPYRUVATE CARBOXYKINASE mRNA

Lea RESHEF, David FALIKS, Victoria BENTOR-GETTER and Gad GLASER
Department of Biochemistry and Cellular Biochemistry, Hebrew University-Hadassah Medical School, PO Box 1172, Jerusalem, Israel

Received 4 October 1978

1. Introduction

It has been repeatedly shown that both protein synthesis and ribosomal aggregation are facilitated by amino acids [1–3] or glucose feeding [3] while fasting or diabetes lead to a considerable disaggregation of polyribosomes [1–4]. It has been demonstrated [5] that both free and membrane bound polysomes undergo disaggregation in liver cells of fasted animals.

Rat liver cytosol P-enolpyruvate carboxykinase is induced by fasting and its intracellular level rapidly declines after glucose feeding [6–9] or administration of insulin to diabetic rats [7–8]. This occurs despite the enhancement of total protein synthesis in response to glucose feeding [10], suggesting a specific decrease in the amount of functional mRNA coding for the enzyme. Indeed, we have recently shown that glucose feeding caused a marked decline of the translatable activity of the mRNA for P-enolpyruvate carboxykinase as determined by an heterologous cell-free system [9]. However, when measured separately, the translational activity of this message from total liver RNA declined twice as fast as that from polysomal RNA [9]. It was assumed that glucose feeding promoted the aggregation of liver ribosomes [3,4], thereby enabling the P-enolpyruvate carboxykinase message to become more effectively expressed prior to its degradation. To test this assumption, the synthesis of P-enolpyruvate carboxykinase in vitro by postmitochondrial liver supernatants was determined at short intervals after glucose feeding. In addition, template activity of total and polysomal RNA was determined before and shortly after glucose feeding. The results of these experiments and their implications are discussed here.

Abbreviations P-enolpyruvate carboxykinase, phosphoenolpyruvate carboxykinase (GTP) (EC 4 1.1.32); poly(A)$^+$RNA, RNA containing a polyadenylic acid sequence, S-17, postmitochondrial supernatant, SDS, sodium dodecyl sulfate, Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid

2. Experimental

2.1. *Animal and materials*

Week 7 male Sabra rats from the Hebrew University breeding center were used throughout. Rats deprived of food for 24 h were either used as such or tube fed with glucose, 5 g/kg body wt, for the times indicated in the text. Diabetes was produced by injecting alloxan (19 mg/100 g body wt) subcutaneously. Insulin (glucagon-free), a gift of Eli Lilly and Co., Indianapolis, IN. (5 μg/100 g body wt) was injected subcutaneously. Oligo(dT) cellulose type T_3 was from Collaborative Research Inc., Waltham, MA. NCS tissue solubilizer was from Amersham/Searle Corp., SDS from BDH was recrystallized. Sucrose (RNase free) was from Schwartz and Mann. L-[4,5-^{3}H]Leucine (30 Ci/mmol) was from Nuclear Research Centre, Negev, and the wheat germ was a gift of General Mills, Inc.

2.2. *Determination of liver leucine concentration*

This was as in [8]

2.3. *Preparation of postmitochondrial supernatants and polysomes*

Livers were quickly removed, rinsed in ice-cold

sterile solution containing 5 mM Mg acetate, 60 mM KCl, 0.375 M sucrose, in 20 mM Hepes buffer (pH 7.6), blotted and pressed through tissue press porous plate with 1 mm diam. pores. This method was required to preserve the intactness of the polysomes (fig.1). The resultant mash was weighed and homogenized in 3 vol. of the above solution containing 0.4 mg/ml heparin with 3 strokes using a loosely-fitting Teflon homogenizer. The homogenates were spun for 10 min at 17 000 X *g* and the upper 4/5th supernatants were carefully aspirated (denoted S-17). Polysomes were isolated from S-17 by centrifugation through a discontinuous sucrose gradient as in [9].

2.4. *Preparation of poly(A)$^+$RNA*

S-17, 5 ml, or a suspension of polysomes isolated from 10 ml S-17 were phenol extracted as in [11] except that no salt was added to the phenol washes and the LiCl step was carried out for 90 min at 4°C. The eluted poly(A)$^+$RNA from oligo(dT) cellulose column was made 0.2 M in Na-acetate and precipitated in 2 vol. ethanol at −70°C for 90 min. The RNA was then treated as in [11].

2.5. *Polysome profile*

S-17, 20 μl, fraction were diluted to 200 μl with the homogenization solution without sucrose, layered on a 11 ml 0.5–1.5 M linear sucrose gradient in the above buffer and centrifuged for 90 min at 40 000 rev./min in a Beckman SW 41 rotor at 4°C. The gradient was pumped from the bottom and A_{254} was monitored with a UA-4 Isco monitor and 612 ultraviolet recorder

2.6. *S-17 'run-off' protein synthesis*

S-17 'run-off' of protein synthesis was performed as in [10,12] except that the Tris buffer was replaced by Hepes buffer (pH 7.6) in total vol. 0.5 ml.

2.7. *Translation of poly(A)$^+$RNA in the wheat germ cell free system*

The procedure for the preparation of the wheat germ extract and conditions for the translation of exogenous RNA were as in [9].

Quantitation of P-enolpyruvate carboxykinase synthesized in vitro by S-17 'run-off' system or by the wheat germ system was by specific immunoprecipitation followed by SDS–polyacrylamide gel electrophoresis as in [9]. The specific antibody against the enzyme [9] was a gift of Dr F. J. Ballard.

3. Results and discussion

3.1. *Effect of short term glucose feeding on ribosomal aggregation and the extent of protein synthesis*

Feeding of glucose to fasted rats for 30 min induced a shift to larger size polysomes as is demonstrated by the polysome profiled in fig.1. The intracellular pool of leucine was virtually unchanged after glucose feeding (0.96 μmol/g liver ± 0.116 in fasted rats and 0.71 μmol/g liver ± 0.09 in rats 30 min after glucose feeding). In addition, glucose feeding had no effect on the rate of elongation of the polypeptide chain as determined from the rate of release of terminated chains (results not shown). In light of these results and the fact that liver postmitochondrial supernatant is devoid of initiation activity [13,14], we suggest that the enhancement of protein synthesis after glucose feeding (fig.2A) is a result of an increase in the av. no. polypeptide chains synthesized/mRNA molecule. Therefore the observed phenomenon most probably reflects an increase in the translational efficiency in vivo [15].

3.2. *'Run-off' synthesis of P-enolpyruvate carboxykinase by S-17 fraction*

The efficient synthesis of P-enolpyruvate carboxy-

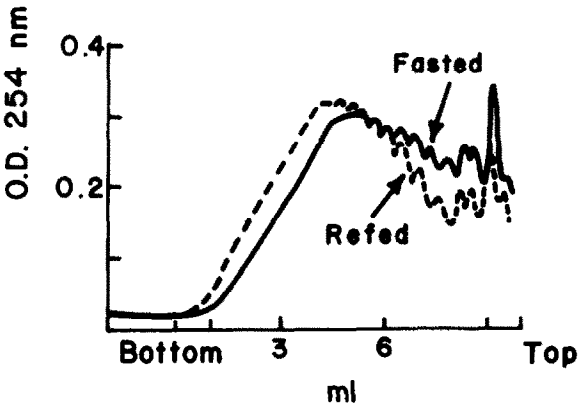

Fig.1. Sucrose gradient analysis of liver polysomes from fasted rats before and 30 min after glucose feeding Conditions for the gradients are as described in section 2

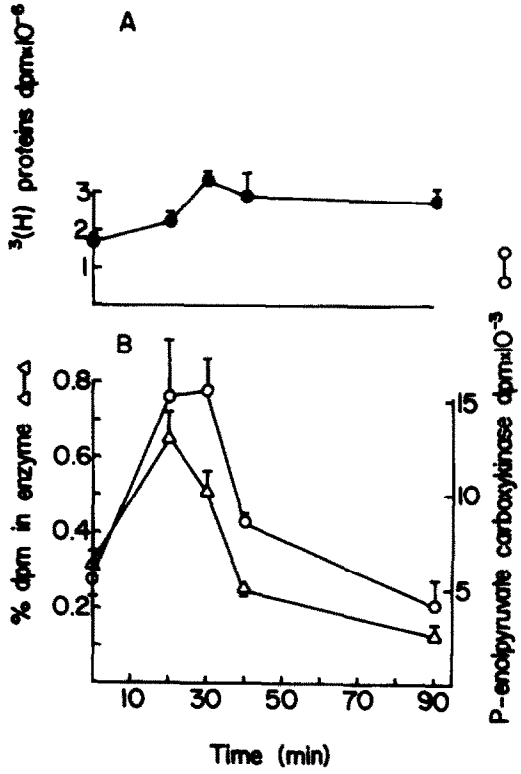

Fig.2 Glucose feeding to fasted rats Time course of effect on protein and P-enolpyruvate carboxykinase synthesis measured by the S-17 'run-off' system in vitro, as described in section 2 Each point represents the average of the incorporation of [^{3}H]leucine into (A) released proteins; (B) P-enolpyruvate carboxykinase (○) dpm in enzyme, (△) % of dpm in enzyme. The bars indicate SEM of 10 animals/point

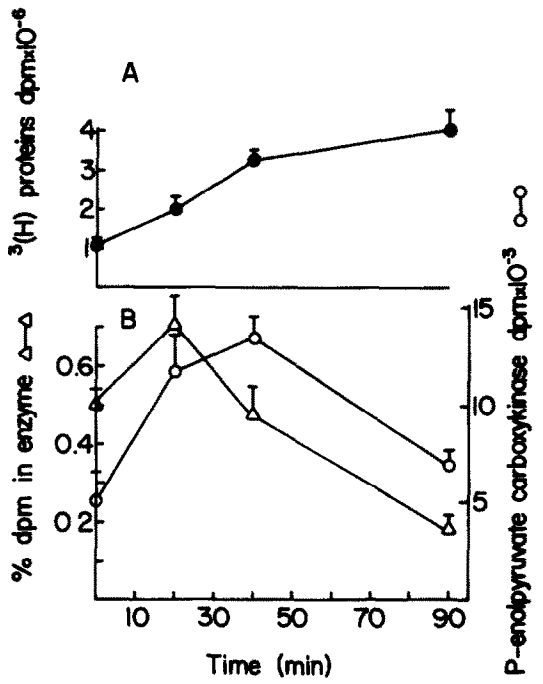

Fig.3. Insulin injection to diabetic rats Time course of effect on protein and P-enolpyruvate carboxykinase synthesis measured by the 'run-off' system in vitro. Each point represents the average of the incorporation of [^{3}H]leucine into (A) released proteins; (B) P-enolpyruvate carboxykinase. (○) dpm in enzyme, (△) % of dpm in enzyme. The bars indicate SEM of 4–5 animals/point

kinase by S-17 has been demonstrated [10,12]. The radioactive peak fractions of the immunoprecipitable synthesis products after SDS–polyacrylamide gel electrophoresis corresponded to the enzyme [10,12]. Using this criterion a 3-fold increase in the synthesis of the enzyme was observed 20 min after glucose feeding (fig.2B). This preceded the increase in total protein synthesis indicated by increased leucine incorporation (fig.2A) While the enhanced synthesis of total proteins persisted for 90 min after glucose feeding, the increase in P-enolpyruvate carboxykinase synthesis was transient, terminating after 30 min. Subsequently, the synthesis of the enzyme abruptly declined in both absolute and relative terms (fig.2B) S-17 from diabetic fed rats treated with insulin exhibited a similar pattern (fig.3) differing in that the synthesis of total proteins increased continuously over the entire 90 min after insulin injection (fig 3A), and the changes in the synthesis of P-enolpyruvate carboxykinase were less abrupt (fig.3B) than those observed after glucose feeding to fasted rats (fig.2B).

3.3. *Translatable activity of poly(A)$^+$RNA from S-17 fraction and polysomes*

Protein synthesis directed by poly(A)$^+$RNA from either the S-17 fraction or polysomes, increased linearly with increasing amounts of RNA up to 5 μg added to the wheat germ system (results not shown), providing an adequate assay of the amount of translatable mRNA. It may be seen from table 1 that the template activity for the synthesis of total proteins of the RNA from both S-17 and polysomes, was not

Table 1
Translatable activity of poly(A)$^+$RNA from S-17 and polysome fractions

Poly(A)$^+$RNA	Treatment of animals	No. of preparations	[^{3}H]Leucine (dpm × 10^{-3}) incorporated into		
			Released proteins	Enzyme	
S-17	fasting	(5)	2010 ± 170	2.98 ± 0.43	$p > 0.10$
	fasting and refeeding	(4)	2160 ± 120	4 55 ± 0.71	
Polysomes	fasting	(4)	3340 ± 497	6.33 ± 1.01	$p < 0$ 05
	fasting and refeeding	(3)	3940 ± 270	11.90 ± 1 96	

Poly(A)$^+$RNA was isolated from S-17 and polysome fractions prepared from livers of rats fasted for 24 h (fasting) and 20 min after glucose feeding (fasting and refeeding). The poly(A)$^+$RNA was assayed for translatable activity as described in section 2. Results are the means in dpm of [^{3}H]leucine incorporated into released proteins and P-enolpyruvate carboxykinase/2.5 μg RNA ± SEM of the no RNA preparations indicated, and corrected for endogenous protein synthesis (900 × 10^{-3} dpm). The translational activity of the S-17 poly(A)$^+$RNA is 2/3rds that of polysomal RNA. Statistical significance of the effect of glucose feeding on enzyme synthesis is indicated

affected by glucose feeding. However, the activity of the poly(A)$^+$RNA from S-17 coding for P-enolpyruvate carboxykinase was not significantly affected by glucose feeding (table 1), while that from the polysomes was elevated about 2-fold (table 1). Since enzyme synthesis by polysomes in the 'run-off' system was increased even more (3-fold) shortly after glucose feeding (fig.2B), we suggest that the results reflect an increase in the translational efficiency (fig.1) concurrent with a shift of P-enolpyruvate carboxykinase mRNA into polysomes (table 1). This provides an explanation for the observed [9] slower decay in the activity of polysomal mRNA relative to total mRNA for the enzyme subsequent to longer glucose feeding.

3.4. *Significance of findings*

In analyzing the present evidence it is instructive to consider recent findings concerning rat liver albumin mRNA indicating that a considerable displacement of this mRNA (~60%) from polysomes is induced by fasting [5]. Despite the fact that albumin mRNA was determined by hybridization [5], it is conceivable that the displaced postribosomal mRNA is not functional. We expected maximally efficient synthesis of P-enolpyruvate carboxykinase in the fasted rat liver. Our findings, that feeding glucose to fasted rats elicited a transient increase in the translatable activity of the polysomal mRNA coding for P-enolpyruvate carboxykinase, imply that only ~60% of the available message for P-enolpyruvate carboxykinase in the fasted rat is functional. The other 40% becomes expressed only upon refeeding the rats with glucose. This conclusion must await further affirmation by direct hybridization experiments. The sub-optimal efficiency of P-enolpyruvate carboxykinase synthesis during fasting probably results from an inherent impaired translational capacity of the liver.

What is the mechanism by which the glucose exerts its effect? We are not in a position to answer this question. However, evidence bearing on this problem has been reported [16,17] showing that intermediates of glucose metabolism such as FDP, are capable of stimulating the initiation step in the process of protein synthesis. The relatively disaggregated polysomes in livers from fasted rats (fig.1) suggest an inhibition of the initiation step This conclusion is in accordance with results obtained in other systems [3,18] where glucose deprivation resulted in the impairment of initiation of protein synthesis.

Acknowledgements

This work was supported by a joint grant from the USA-Israel Binational Science Foundation (1346/77). We especially thank Dr John F. Ballard for generously providing as with antibody to P-enolpyruvate carboxykinase used in this study.

References

[1] Hirsch, C A. and Hiatt, H. H. (1966) J. Biol. Chem. 241, 5936–5940.
[2] Henshaw, E. C., Hirsch, C A., Morton, B. E. and Hiatt, H H. (1971) J. Biol. Chem. 246, 433–446
[3] Pain, M. W. and Henshaw, E. C (1975) Eur. J. Biochem. 57, 335–342.
[4] Wittman, J. S., Lee, K-L. and Miller, O N. (1969) Biochim Biophys. Acta 174, 536–543
[5] Yap, S H., Strair, R. K. and Shafritz, D. A. (1978) J Biol Chem. 253, 4944–4950.
[6] Hopgood, M F., Ballard, F J , Reshef, L. and Hanson, R. W (1973) Biochem. J 134, 445–453.
[7] Tilghman, S. M , Hanson, R W., Reshef, L., Hopgood, M. F. and Ballard, F. J. (1974) Proc Natl Acad. Sci USA 71, 1304–1308
[8] Meyuhas, O., Reshef, L., Gunn, J. M., Hanson, R. W. and Ballard, F. J. (1976) Biochem. J. 158, 1–8.
[9] Kioussis, D., Reshef, L , Cohen, H , Tilghman, S M., Iynedjian, P B., Ballard, F. J. and Hanson, R W (1978) J Biol. Chem. 253, 4327–4332.
[10] Ballard, F. J , Hopgood, M F., Reshef, L , Tilghman, S. M. and Hanson, R W. (1974) Biochem. J. 144, 199–207.
[11] Iynedjian, P. B and Hanson, R W. (1977) J. Biol Chem. 252, 8398–8400.
[12] Iynedjian, P. B. and Hanson, R. W. (1977) J. Biol. Chem. 252, 655–662.
[13] Schreier, M. F. and Staehelin, T (1973) J Mol. Biol. 73, 329–349.
[14] Mathews, M. B. and Osborn, M (1974) Biochim. Biophys. Acta 340, 147–152
[15] Palmiter, R. D. (1975) Cell 4, 188–197.
[16] Lenz, J R , Chatterjee, G. E., Maroney, P. A. and Baglioni, C. (1978) Biochemistry 17, 80–87.
[17] Freudenberg (Giloh), H. and Mager, J. (1975) Biochim. Biophys. Acta 414, 293–308.
[18] David, M and Avi-Dor, Y. (1975) Biochem. J. 150, 405–411