GLUCOSE-DEPENDENT STIMULATION OF PROTEIN SYNTHESIS IN CULTURED HEART MUSCLE CELLS

Possible involvement of the pentose phosphate pathway

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1. Introduction

The irreversible damage inflicted by lasting anoxia to the myocardium has been ascribed [1] to a depletion of the ATP pool below the concentration that is essential for the structural and functional integrity of the cells. It was proposed [2] that lack of ATP might shift the delicate balance which exists between the synthesis and the breakdown of proteins in the heart tissue in the favour of the latter and may thus be one of the underlying causes for the irreversible damage. In the isolated, perfused heart the damage caused by anoxia was prevented [3] by including glucose into the perfusion medium. This beneficial effect of glucose was attributed to the maintenance of the ATP concentration in the cells. Cultured heart muscle cells were used to investigate the effect of glucose on protein synthesis [4]. Since glucose was found to stimulate protein synthesis in the anaerobic heart cells also under conditions when it caused no rise in the ATP concentration level, we suggested that in addition to ATP generation also some other factors must be involved in the 'glucose-effect'. In cell free extracts certain phosphorylated sugars stimulated the initiation and elongation of the polypeptide chain [5–8]. Prompted by these findings we have investigated whether products formed from glucose through the glycolytic pathway and/or the pentose phosphate pathway (PPP) could be responsible for the glucose-effect observed in anaerobic heart muscle cells. The results indicate the involvement of the PPP in the glucose-dependent stimulation of protein synthesis.

2. Materials and methods

2.1. *Preparation of cultured cells and assay conditions*

Heart cells obtained from 3-day-old newborn rats (Wistar strain) were cultured in Petri dishes according to [9] with the modifications in [4]. 3-Day-old cultures were used for the assay of protein synthesis and various metabolic activities. The assay conditions and the assay media were as in [4] except that KCN was used to inhibit the respiration of the cells. Under the nitrogen atmosphere used previously for this purpose, traces of oxygen (detected by release of $^{14}CO_2$ from [6-^{14}C]glucose) unavoidably infiltrated the system during incubation. This was undesirable, since we intended to study specifically the effect of the intermediates formed in glycolysis and the PPP on protein synthesis. A rather high concentration of KCN (3–5 mM) was found to be necessary to suppress side effects of oxygen, such as the 'Pasteur effect' [10].

2.2. *Determination of glycolytic products, ATP and pentose phosphate pathway activity*

Glucose uptake and lactate plus pyruvate production by the cells were determined according to [11,12] by adding [U-^{14}C]glucose (1.5×10^5 cpm/μmol) to the assay medium, separating by paper chromatography the labelled glucose from the products formed, and assessing the distribution of radioactivity on the chromatogram. In the $HClO_4$-soluble fraction of the cells glucose 6-phosphate (G-6-P) was determined according to [13]; fructose 1,6-diphosphate (F-1,6-diP) by a modification of [14] and ATP according to [15]. The amount of $^{14}CO_2$ released during the incubation of the cells with [1-^{14}C]glucose (4.0×10^5 cpm/μmol)

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was determined according to [16] and it served as a measure for the activity of the pentose phosphate pathway.

2.3. *Estimation of the rate of protein synthesis, counting of radioactivity, protein determination; materials*

All these estimations were performed as in [4]. Radioactive materials and other chemicals were purchased from the sources in [4].

3. Results

According to [17] the activity of the PPP is controlled by the availability of $NADP^+$ and hence by the rate of reoxidation of NADPH. The latter rate will be necessarily limited when respiration is suppressed. There are several ways known, however, to stimulate the reoxidation of NADPH. In case that the PPP is either directly or indirectly involved in the regulation of protein synthesis one could expect that stimulation of the PPP in non-respiring, glycolyzing heart muscle cells will also enhance the rate of protein synthesis. This assumption was put to a test: In erythrocytes low concentrations of the auto-oxidizable dye, methylene blue, was reported to stimulate the PPP activity [18]. Under the conditions used for the assay of the PPP-activity and protein synthesis in cultured heart muscle cells, however, this dye did not produce reproducible results. Therefore, its use was abandoned. Pyruvate was shown to stimulate strikingly the PPP activity in erythrocytes and in certain types of tumor cells [19–21]. This stimulation was shown to be due to transfer of reducing equivalents from NADPH to pyruvate by lactate dehydrogenase (LDH, EC 1.1.1.27) which possessed sufficient affinity for NADPH to account for the observed stimulation of the PPP-activity. In heart- or skeletal muscle homogenates an acceleration of the PPP resulted from the addition of ribose-5-phosphate (R-5-P) to the system [22]. Also in this case, stimulation of NADPH oxidation was found to be the underlying cause for the effect. The mechanism suggested involves the formation of glyceraldehyde 3-phosphate from R-5-P through the PPP and reduction of the former compound catalyzed by the NADPH-dependent glyceraldehyde 3-phosphate dehydrogenase. When pyruvate was added to the respiration-inhibited, glycolyzing, heart muscle cells, then, like in erythrocytes or tumor cells, the PPP-activity significantly increased (table 1). Since R-5-P does not permeate the plasma membrane we tested the effect of ribose and found that it stimulated the PPP-activity, possibly after conversion to R-5-P. Ribose is well known to be metabolized by the heart muscle tissue [23]. Another important point which emerged from the experiments shown in table 1 was that though both the above two substances increased the rate of protein synthesis pyruvate strongly inhibited, whereas ribose moderately stimulated the glycolysis. Thus, apparently the rate of the PPP rather than that of the glycolytic flux is limiting the protein synthesis. The experiment in table 2 was conducted to find out whether pyruvate or ribose by themself would affect protein synthesis. It can be seen from table 2 that in presence of 5 mM KCN the omission of glucose caused severe depletion in the ATP content of the cells but this could be prevented by reducing the respiratory inhibitor to 1 mM. However, neither

Table 1
Effect of pyruvate and ribose on glycolysis, PPP-activity and glucose-dependent stimulation of protein synthesis

Additions	Glucose consumed[a]	Lactate and pyruvate produced[a,b]	ATP[c]	$[^{14}C]CO_2$[c,d]	$[^3H]$Leucine incorpn.[c]
None	0.9	1.8	16.1	5.0	1.8
Pyruvate (5 mM)	0.4	0.7	18.0	9.0	4.0
Ribose (50 mM)	1.2	2.4	17.0	10.0	3.3

[a] μmol/mg protein; [b] the chromatographic method did not separate the two labelled products; [c] nmol/mg protein; [d] released from [1-^{14}C]glucose

Cells were incubated for 30 min at 37°C as in section 2. The standard medium of incubation contained glucose (5 mM) and KCN (5 mM). Other additions were as indicated. The data given are representative values: Each experiment was repeated at least 5 times

Table 2
Effect of pyruvate and ribose on protein synthesis in the absence of glucose

Additions	KCN (mM)	ATP[a]	[^{3}H]Leucine incorpn.[a]
None	5.0	3.2	0.4
Pyruvate	5.0	3.7	0.4
	1.0	15.0	0.4
Ribose	5.0	4.5	0.5
	1.0	17.0	0.5

[a] nmol/mg protein

Glucose was omitted from the standard medium of incubation. Other additions were as indicated. Conditions of the experiment were as in table 1.

pyruvate nor ribose affected the rate or protein synthesis in the absence of glucose – not even when sufficient ATP was generated by the residual respiratory activity.

The causal relation between the activity of the PPP and the rate of protein synthesis was also indicated by the finding (fig.1) that both of the above parameters were affected in a similar manner by variations in the pyruvate concentration. Moreover, also the time-course of $^{14}CO_2$ release from [1-^{14}C]glucose and that of the incorporation of [^{3}H]leucine showed remarkable similarity (fig.2).

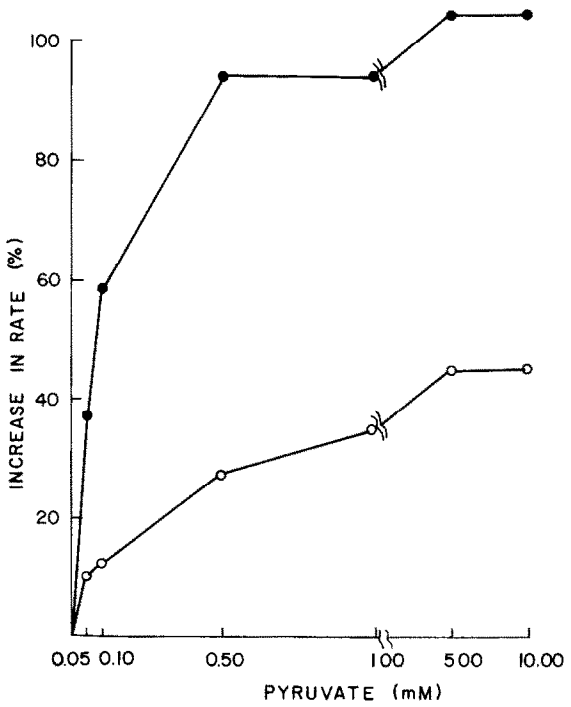

Fig.1. Protein synthesis and PPP-activity as a function of the pyruvate concentration. Conditions of incubation as in table 1 with 5 mM glucose added to the medium: (○) $^{14}CO_2$ release from [1-^{14}C]glucose; (●) [^{3}H]leucine incorporation.

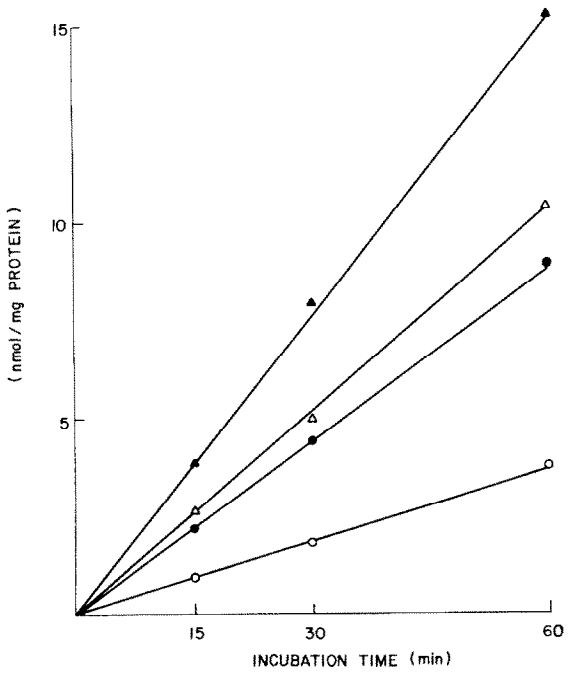

Fig.2. Kinetics of protein synthesis and PPP-activity in the presence and absence of pyruvate (5 mM). Conditions of incubation as in fig.1. (○,●) [^{3}H]Leucine incorporation; (△,▲) $^{14}CO_2$ release from [1-^{14}C]glucose; open symbols, no pyruvate added; shaded symbols, with 5 mM pyruvate added.

Though apparently it is not related to protein synthesis, we attempted to locate the step(s) in glycolysis which were affected by added pyruvate. Refering to the techniques used (see section 2) to separate the products of glycolysis, dilution of the labelled products by the added unlabelled pyruvate could not account for the inhibition, since pyruvate and lactate were not separated on the chromatogram. Moreover, also the uptake of glucose was inhibited. Heart muscle LDH is known to be inhibited by pyruvate [24]. Inhibition of LDH would limit the regeneration of NAD^+ and thus also the activity of glyceraldehyde 3-phosphate dehydrogenase. The resulting pattern would be similar to that obtained by inhibiting the latter enzyme with iodoacetate: i.e., accumulation of F-1,6-diP would be expected to occur. However, as seen from table 3 in contrast to iodoacetate the addi-

Table 3
Effect of pyruvate and/or iodoacetate on the concentration of the glycolytic intermediates

Additions	G-6-P[a,b]	F-1,6-diP[a,c]
Glucose	2.6	13.3
+ pyruvate	1.3	7.6
+ iodoacetate	1.8	24.0
+ iodoacetate + pyruvate	2.2	23.0

[a] nmol/mg protein; [b] glucose 6-phosphate; [c] fructose-1,6-diphosphate

Glucose (5 mM) and KCN (5 mM) were added to the standard medium of incubation. When indicated pyruvate (5 mM) and/or iodoacetate (5 μM) were also added. Conditions were otherwise as in table 1

tion of pyruvate reduced the intracellular concentration of F-1,6-diP. Since in presence of pyruvate also the concentration of G-6-P was lowered it is reasonable to assume that pyruvate inhibited the transport of glucose or its conversion to G-6-P by hexokinase, rather than the LDH.

4. Discussion

The most important point emerging from this work is the demonstration of a certain interrelation in the cultured heart muscle cells between protein synthesis and PPP-activity. This finding suggests that a product of the PPP might be involved in the regulation of protein synthesis. The role of phosphorylated sugars in the regulation of protein synthesis has been implicated in the restoration of protein synthesis to lysates prepared from glucose-starved-reticulocytes [5], and also to lysates in which protein synthesis was impaired by heme deficiency or by adding oxidized glutathion [7]. The stimulatory effect of phosphorylated sugars was also demonstrated in a variety of other mammalian cells [8]. At this stage of investigation it is not possible to pinpoint the product of the PPP to which the observed effects could be ascribed. It may be relevant in this context to recall that ribose, which after being converted to R-5-P, is expected to produce the intermediates of the PPP, was not able in the absence of glucose to stimulate protein synthesis, just as pyruvate was not able to do so. Thus, the common denominator between the effect of pyruvate and ribose is plausibly the facilitation of the reoxidation of NADPH produced in the first two steps of the PPP. Therefore, either the regulation of the balance between NADPH and $NADP^+$ is of importance for some step in protein synthesis or alternatively the concerted action of a glycolytic intermediate (e.g., G-6-P) and of a PPP-intermediate is involved in the 'glucose effect'.

With regard to the possible physiological implications of these findings it is noted that according to [25] during the period of recovery from experimentally induced heart infarct not only the rate of protein synthesis was increased in the vicinity of the damaged site but also PPP-activity was found to be enhanced up to 30-fold. The latter activity returned to its normal level after healing of the damaged tissues. It is tempting to speculate about the possibility that the above intimate connection between PPP-activity and tissue regeneration is not restricted to heart infarct. Activation of the PPP might more generally accompany events in which accelerated cell growth necessitates an increment in the rate of protein synthesis.

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References

[1] Jennings, R. B. (1969) Am. J. Cardiol. 24, 753–765.
[2] Wollenberger, A., Onnen, K., Hinterberger, U., Rabitzsch, G. and Kleitke, B. (1971–1972) Cardiology 56, 48–64.
[3] Hearse, D. J. and Chain, E. B. (1972) Biochem. J. 128, 1125–1135.
[4] David, M. and Avi-Dor, Y. (1975) Biochem. J. 150, 405–411.
[5] Giloh (Freudenberg), H. and Mager, J. (1975) Biochim. Biophys. Acta 414, 293–308.
[6] Giloh (Freudenberg), H., Schochot, L. and Mager, J. (1975) Biochim. Biophys. Acta 414, 309–320.
[7] Ernst, V., Levin, D. H. and London, I. M. (1978) J. Biol. Chem. 253, 7163–7172.
[8] Lenz, J. R., Chatterjee, G. E., Maroney, P. A. and Baglioni, C. (1978) Biochemistry 17, 80–87.
[9] Harar, I. and Farley, B. (1963) Expl. Cell Res. 29, 451–455.
[10] Krebs, H. A. (1972) Essays Biochem. 8, 1–34.
[11] Vernon, R. G. and Walker, D. G. (1972) Biochem. J. 127, 521–529.

[12] Riley, M. V. (1968) Anal. Biochem. 22, 341–345.
[13] Hohorst, H. J., Kreutz, F. H. and Bücher, Th. (1959) Biochem. Z. 332, 18–46.
[14] Gracy, R. W., Lacko, A. G., Brox, L. W., Adelman, R. C. and Horecker, B. L. (1970) Arch. Biochem. Biophys. 136, 480–490.
[15] Stanley, P. E. and Williams, S. G. (1969) Anal. Biochem. 29, 381–392.
[16] Warshaw, J. B. and Rosenthal, M. D. (1972) J. Cell. Biol. 52, 283–291.
[17] Eggleston, L. W. and Krebs, H. A. (1974) Biochem. J. 138, 425–435.
[18] Szeinberg, A. and Marks, P. A. (1961) J. Clin. Invest. 40, 914–924.
[19] Beutler, E. and Guinto, E. (1974) Enzyme 18, 7–18.
[20] Sagon, A. L. jr, Balcerzak, S. P. and Metz, E. N. (1975) Blood 45, 49–54.
[21] Shrivastava, G. C. and Mallick, L. (1967) Ind. J. Cancer 4, 38–41.
[22] Glushankov, P. E., Epifanova, Yu. E. and Kolotilova, A. I. (1976) Biokhimya 41, 1788–1790.
[23] Jolley, R. L., Cheldelin, V. H. and Newburgh, R. W. (1958) J. Biol. Chem. 233, 1289–1294.
[24] Kaplan, N. O. and Everse, J. (1972) Adv. Enz. Reg. 10, 323–335.
[25] Gudbjarnason, S., Cowan, Ch., Braasch, W. and Bing, R. J. (1967) Cardiologia 51, 148–159.