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Stimulation of Protein Synthesis in Cultured Heart Muscle Cells by Glucose

By M. DAVID and Y. AVI-DOR
Department of Biology, Technion–Israel Institute of Technology, Haifa, Israel

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Glucose stimulated the rate of incorporation of [^{3}H]leucine into $HClO_4$-insoluble fraction of cultured rat heart muscle cells under both aerobic and anaerobic conditions. In the aerobic system the incorporation proceeded at a constant rate during 3h of incubation with and without glucose whereas in the anaerobic system the incorporation ceased after approx. 60min and could be renewed only by the addition of glucose. No correlation was found to exist between the above effect of glucose on protein synthesis and glucose-dependent changes in the intracellular ATP concentration. The extent of the stimulation of protein synthesis was related to the concentration of glucose. The effect of glucose was suppressed by cycloheximide but was not affected by actinomycin D. Glucose had no effect on the rate of transport of α-aminoisobutyric acid. Mannose also stimulated [^{3}H]leucine incorporation. Substances that did not produce lactate were ineffective. Iodoacetate inhibited the stimulatory effect of glucose, but pyruvate, which by itself had no appreciable stimulatory action, relieved the inhibition induced by iodoacetate. There was no concomitant change in the concentration of ATP when iodoacetate inhibition was reversed by pyruvate. L-Lactate or other intermediates of energy metabolism could not relieve the inhibitory effect of iodoacetate.

A decrease in the blood supply to the myocardium induces both local hypoxia and deficiency in the exogenous substrates (i.e. glucose, amino acids and fatty acids). In an early phase of the ischaemic condition there is already a cessation of the contraction of the myocardium, but only prolonged ischaemia leads to irreversible changes and to cell death. (For a review of the sequence of events, see Jennings, 1969; Jennings *et al.*, 1969.) This early-phase malfunction of the heart is generally attributed to a lack of ATP and an increase in the intracellular acidity whereas the cause of irrreversible damage is ascribed to depletion of the ATP pool below the concentration that is essential for the maintenance of the structural and functional integrity of the cells (Jennings, 1969). According to the above concept, the maintenance of ATP concentration during anoxia should be capable of preventing irreversible damage. Indeed, it was found (Weissler *et al.*, 1968; Hearse & Chain, 1972) that when isolated hearts were perfused with an anoxic buffer solution, contractions stopped whether or not glucose was included in the perfusate. However, on subsequent reoxygenation, the extent and the rate of recovery were greatly improved by the presence of glucose during the anaerobic phase of the experiment. Nonetheless, the evidence for a causal relation between ATP maintenance and the beneficial effect of glucose is only circumstantial and there is an indication of the involvement of factors other than ATP in the effect of glucose on the survival of heart muscle. For example, Wildenthal (1973) reported that glucose is needed for the long-term survival of foetal mouse hearts in organ culture under conditions when there are ample alternative energy sources available for ATP synthesis. Others, who studied the effect of anoxia or ischaemia on the myocardium, focused their attention on the nature of the biochemical lesion that leads to cell death. For example, Wollenberger *et al.* (1971–72) suggested that the underlying cause of irreversible damage to the myocardium might be a perturbation in the delicate balance that exists between the rate of synthesis and breakdown of proteins in favour of the latter.

The present work was prompted by Wollenberger's suggestion. By using cultured heart muscle cells as a model system for the myocardium, we investigated the effect of glucose on protein synthesis. It was found that glucose markedly stimulated protein synthesis under anaerobic as well as aerobic conditions and that this glucose effect cannot be accounted for by a regulation of the intracellular ATP pool.

Experimental

Animals

Rats of the Wistar strain were used throughout the investigation.

Materials

Special materials were obtained from the following sources: actinomycin D, firefly lanterns (desiccated tails), lactate dehydrogenase from rabbit muscle, type I (L-lactate–NAD^+ oxidoreductase, EC 1.1.1.27), from Sigma Chemical Co., St. Louis, Mo., U.S.A.; cycloheximide (actidione) and Triton X-100 from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K.; trypsin (EC 3.4.21.4; fraction 1-3000 from hog pancreas) from National Biochemicals Corp., Cleveland, Ohio, U.S.A.; crystalline sodium penicillin G and dihydrostreptomycin sulphate were from RAFA Laboratories, Jerusalem, Israel; horse serum (membrane-filter sterilized) from Flow Laboratories, Irvine, Ayrshire, U.K. [U-^{3}H]Leucine (250mCi/mmol), [4,5-^{3}H]lysine (19Ci/mmol), [U-^{3}H]phenylalanine (1 Ci/mmol) and [5,6-^{3}H]uridine (51 Ci/mmol) from The Radiochemical Centre, Amersham, Bucks., U.K. and α-[U-^{3}H]aminoisobutyric acid (1mCi/0.928mg) from New England Nuclear Corp., Boston, Mass., U.S.A.

Other chemicals were of analytical grade.

Preparation of the cultured cells

The hearts of 2–3-day-old newborn rats were quickly removed and cultured in 60mm Petri dishes essentially by the method of Harary & Farley (1963) except that the growth medium contained 20% (v/v) horse serum instead of human and foetal-calf serum. Approx. 5×10^6 cells were seeded into each plate. Cultures (2–3 days old) containing cells in an amount equivalent to 0.5–1.0mg of protein were used.

Media

The trypsin solution, saline A, Hank's saline and nutrient solution were prepared as described by Harary & Farley (1963).

Assay conditions

The growth medium was decanted and the cells were washed twice with saline A (37°C) not containing glucose (–glucose). To each plate (unless otherwise stated) 3.9ml of the standard medium was added. The standard medium contained in a proportion of 3:5:1 (by vol.) Hank's saline (–glucose), nutrient solution (–glucose, –leucine) and horse serum dialysed for 24h against cold saline A (–glucose). Glucose or other metabolites were added in a volume of 40μl as indicated. The incubation was carried out at 37°C with shaking (40rev./min). The composition of the gas phase was CO_2+N_2 or atmosphere (5:95). The assay was started by the addition of the desired radioactive isotope to the medium and was terminated by the removal of the medium and three successive washings with cold (0°C) saline A (–glucose).

Analytical methods

Determination of the intracellular ATP and the incorporation of radioactivity into the acid-insoluble fraction. The medium was decanted, the cells were washed and 1.0ml of cold (0°C) 0.3M-$HClO_4$ was added to each plate and left in contact with the cells at 0°C for 20min. After decanting, the plate was swirled with 1.0ml of $HClO_4$ and 0.1ml of the combined $HClO_4$ extract was used for ATP determination by the method of Stanley & Williams (1969). Water (1.0ml) was added to the acid-extracted cells and the latter were scraped off the plates with a rubber 'policeman'. The cell suspension was transferred into a test tube and the residual cells, still adhering to the plate, were removed by a repetition of the above procedure. The joint cell suspension was sonicated in an MSE sonicator for 2min at 0°C and treated with 0.2ml of 10% (w/v) of sodium dodecyl sulphate to solubilize the cells. For the determination of the radioactivity, 0.5–1.0ml of the solubilized cells was transferred into a vial containing 10.0ml of 'Triton X-100–Toluene Scintillator' (Turner, 1969).

Counting of radioactivity was performed in a model 3002 Packard liquid-scintillation spectrophotometer.

Measurement of the uptake of α-[^{3}H]aminoisobutyric acid. Plates were incubated in the standard medium with α-[^{3}H]aminoisobutyric acid as described above under 'Assay conditions'. After the termination of the assay and the washings, the cells were resuspended and solubilized as described in the previous section except that no $HClO_4$ was added before the cells were scraped off.

Protein determination. A portion (0.3–0.5ml) of the solubilized cells was used for protein determination by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Determination of L-*lactate.* The amount of L-lactate produced by the cells and released into the medium was measured as described by Hohorst *et al.* (1959) in a Cary model 118 recording spectrophotometer.

Results

Effect of glucose on the incorporation of [^{3}H]leucine and on the intracellular ATP concentration

Figs. 1(*a*) and 1(*b*) show the effect of glucose on the time-course of incorporation of [^{3}H]leucine into the $HClO_4$-insoluble fraction of cultured heart muscle cells and on the intracellular ATP concentration. The experiment was conducted either under anaerobic (Fig. 1*a*) or aerobic (Fig. 1*b*) conditions. In both cases glucose markedly stimulated the initial rate of

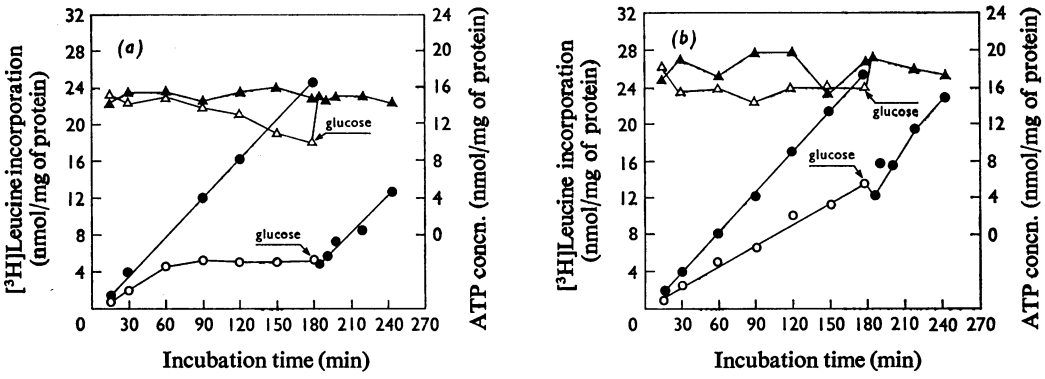

Fig. 1. *Effect of glucose on the rate of protein synthesis and on the ATP concentration in cultured rat heart muscle cells*

Cultured rat heart muscle cells were incubated anaerobically (*a*) or aerobically (*b*) in the standard medium, with (●, ▲) or without (○, △) 5mM-glucose in the presence of 40μM-[^{3}H]leucine (1.1×10^4c.p.m./nmol). At indicated times, one of the plates was removed and used for the determination of the radioactivity incorporated into the acid-insoluble fraction (○, ●) and the concentration of the intracellular ATP (△, ▲). For details see the Experimental section.

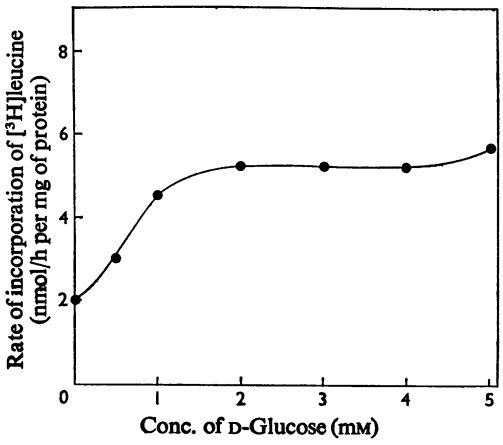

Fig. 2. *Effect of glucose concentration on the rate of protein synthesis*

Cells were incubated at 37°C for 60min under anaerobic conditions in the standard medium containing [^{3}H]leucine as in Fig. 1 and various concentrations of glucose. Incorporation of radioactivity into the acid-insoluble fraction was measured.

Table 1. *Effect of glucose on the incorporation of various amino acids into the acid-insoluble fraction*

Cells were incubated for 30min at 37°C with the standard medium to which also 40μM-leucine was added. The following amino acids were labelled: leucine (1.1×10^4c.p.m./nmol), lysine (1.6×10^3c.p.m./nmol), phenylalanine (4.1×10^3c.p.m./nmol). The incorporation of radioactivity into the acid-insoluble fraction was determined as described in the Experimental section.

	^{3}H-labelled amino acid incorporation (nmol/mg of protein)	
Amino acid	No glucose	Glucose (5mM)
Leucine	4.5	5.4
Lysine	4.1	5.1
Phenylalanine	2.2	2.8

[^{3}H]leucine incorporation without affecting the initial concentration of ATP. In the aerobic experiment (Fig. 1*b*) the rate of [^{3}H]leucine incorporation remained constant whether or not glucose was included in the assay medium. Under anaerobic conditions (Fig. 1*a*) a constant rate of incorporation was maintained only in the presence of glucose. In the glucose-deficient medium the rate of incorporation started to decline before any appreciable change was noted in the ATP concentration and the incorporation stopped when the ATP concentration in the cells was still approx. 95% of its initial value. When glucose was added after cessation of the [^{3}H]leucine incorporation, the latter was renewed at the same rate as shown by those cells that contained glucose from the beginning of the assay.

Although, as shown above, glucose was found to exert its effect on [^{3}H]leucine incorporation under aerobic as well as under anaerobic conditions, the following experiments were always conducted under anaerobic conditions. Our choice was influenced by the consideration that, in the absence of respiration, the number of the products that might be formed from glucose and might be involved in the glucose

Table 2. *Effect of cycloheximide and of actinomycin D on the rate of [^{3}H]leucine and [^{3}H]uridine incorporation*

Cells were incubated for 30min at 37°C with the standard medium containing also either 40μM-[^{3}H]leucine (1.1×10^4c.p.m./nmol) or 10μM-[^{3}H]uridine (4.3×10^4c.p.m./nmol). In the experiment with uridine 40μM unlabelled leucine was also present. Incorporation of radioactive material into the acid-insoluble fraction was determined as described in the Experimental section.

Additions	[^{3}H]Leucine incorporation (nmol/30min per mg of protein)		[^{3}H]Uridine incorporation (nmol/30min per mg of protein)	
	No glucose	Glucose (5mM)	No glucose	Glucose (5mM)
None	2.3	3.5	0.6	0.9
Cycloheximide (50μM)	0.2	0.2	—	—
Actinomycin D (20μg/ml)	2.3	4.0	0.1	0.1

effect on protein synthesis is necessarily much more limited than in the aerobic system.

Effect of glucose concentration on the rate of [^{3}H]-leucine incorporation

The effect of the concentration of glucose on the rate of [^{3}H]leucine incorporation was studied (Fig. 2). The curve which relates the rate of incorporation to the glucose concentration showed a sigmoidal shape. The incorporation proceeded at a maximum rate when the glucose concentration was above 2mM.

Effect of glucose on the rate of incorporation of [^{3}H]lysine and [^{3}H]phenylalanine

To ascertain whether the glucose-induced acceleration of the incorporation of [^{3}H]leucine indeed reflected an effect of glucose on the rate of protein synthesis, we tested whether a similar effect could also be demonstrated with [^{3}H]lysine or [^{3}H]phenylalanine. As shown in Table 1, glucose stimulated the rate of incorporation of the latter two amino acids into the acid-insoluble fraction and the stimulation was similar in magnitude to that for [^{3}H]leucine.

Effect of cycloheximide and of actinomycin D on the incorporation of [^{3}H]leucine

Cycloheximide, which is a potent inhibitor of protein synthesis in eukaryotic cells (Ennis & Lubin, 1964), was found to inhibit [^{3}H]leucine incorporation both in the presence and the absence of glucose (Table 2). In contrast, the inhibitor of DNA-dependent RNA synthesis, actinomycin D (Hartmann *et al.*, 1968), when used at a concentration that suppressed the incorporation of [^{3}H]uridine into the $HClO_4$-insoluble fraction by 80%, affected neither the basic rate of leucine incorporation nor the glucose-stimulated incorporation.

Effect of glucose on the rate of uptake of α-aminoisobutyric acid

Since it is possible that glucose stimulates protein synthesis by affecting amino acid transport, its effect on transport was investigated. For this purpose the non-metabolized amino acid analogue, α-aminoisobutyric acid, was used. This analogue is transported but is not incorporated into proteins. As seen from Fig. 3, the rate of uptake of this analogue was not influenced by glucose. This finding is in accord with the results of Dunand *et al.* (1972), who similarly did not observe stimulation of the uptake of α-aminoisobutyric acid by adding glucose to the medium of incubation of cultured beating heart muscle cells.

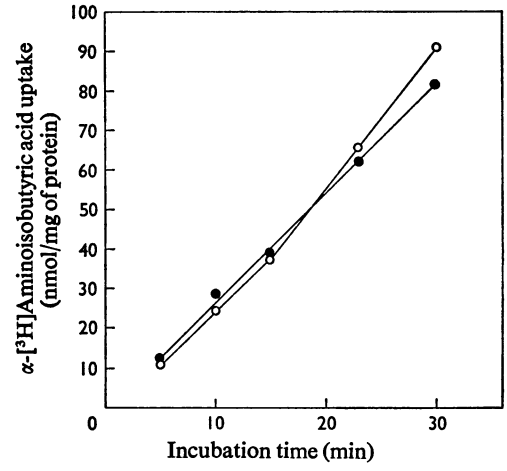

Fig. 3. *Effect of glucose on the uptake of α-aminoisobutyric acid*

Cells were incubated anaerobically. The incubation medium contained: 8vol. of Hank's saline or Hank's saline from which glucose has been omitted, 1vol. of dialysed horse serum and 1mM-α-[^{3}H]aminoisobutyric acid (0.5×10^6c.p.m./μmol). At the times indicated the respective plates were removed and the amount of α-aminoisobutyric acid associated with the cells was determined as described in the Experimental section. ○, No glucose; ●, 5mM-glucose.

Table 3. *Effect of glucose on protein degradation*

Cell proteins were prelabelled with [^{3}H]leucine by incubating the cells under aerobic conditions in the standard medium containing 40μM-[^{3}H]leucine (1.2×10^4 c.p.m./nmol) and 5mM-glucose for 2h. After washing three times with saline A (−glucose) at 37°C, the prelabelled cells were incubated anaerobically for 60min at 37°C in the standard assay medium in the presence of 50μM-cycloheximide with or without 5mM-glucose. The radioactivity associated with the acid-insoluble fraction was determined before and after the incubation period.

Additions	[^{3}H]Leucine in the acid-insoluble fraction (nmol/mg of protein)	
	At zero time	After 60min
None	13.9	12.6
Glucose (5mM)	13.9	12.7

Effect of glucose on protein degradation

Inhibition of the degradation of cellular proteins possessing a high turnover rate, might be expressed as an apparent increase in the rate of incorporation of labelled amino acids into the $HClO_4$-insoluble fraction. To test whether glucose inhibited the breakdown of proteins, cells prelabelled with [^{3}H]leucine were used. The conditions for the assay of protein degradation were the same as for the measurement of [^{3}H]leucine incorporation, except that cycloheximide was also added. The addition of this inhibitor of protein synthesis served to prevent the reincorporation of [^{3}H]leucine released from the prelabelled cellular proteins during degradation. As shown in Table 3, the rate of release of the radioactive material from the prelabelled cells was only about 15–20% of the rate at which [^{3}H]leucine was incorporated into the protein fraction. Glucose was found to have no measurable effect on the rate of protein degradation.

Specificity of the glucose effect

Table 4 shows that among the compounds tested for their effect on [^{3}H]leucine incorporation, only mannose was found to have a stimulatory action comparable with that of glucose. It is relevant in this context to note that only the two active compounds, glucose and mannose, gave rise to lactate after the incubation. Fructose and glycerol were ineffective in this respect as were the two analogues of glucose, 2-deoxyglucose and 3-*O*-methylglucose. It is noteworthy that a decrease in ATP concentration by more than 50%, which took place in the presence of 3-*O*-methylglucose, had no effect on the basic rate of protein synthesis. The latter became affected only when the ATP depletion was more severe, as for example, with 2-deoxyglucose.

Table 4. *Comparison between the effect of various sugars and related compounds on protein synthesis, lactate formation and on the concentration of ATP*

Cells were incubated for 2h at 37°C with the standard medium to which 40μM-[^{3}H]leucine (1.1×10^4c.p.m./nmol) was also added. The incorporation of radioactive material into the acid-insoluble fraction, the amount of lactate produced and the intracellular ATP concentration were measured as described in the Experimental section.

Addition	[^{3}H]Leucine incorporation (nmol/mg of protein)	ATP (nmol/mg of protein)	Lactate (μmol/mg of protein)
None	10.0	17.0	0.6
Glucose	20.0	16.0	2.8
Mannose	16.7	15.0	2.4
Fructose	11.2	16.9	0.8
Glycerol	10.8	18.8	0.9
3-*O*-Methylglucose	11.7	7.8	0.9
2-Deoxyglucose	1.5	3.0	0.6

Effect of iodoacetate on [^{3}H]leucine incorporation

To find out which steps in glycolysis are essential for the glucose effect on protein synthesis, we blocked the step catalysed by glyceraldehyde 3-phosphate dehydrogenase with iodoacetate.

In Table 5 two representative experiments are given. It can be seen that in the absence of glucose, iodoacetate slightly inhibited the basic amount of [^{3}H]leucine incorporation but caused no appreciable change in the intracellular ATP concentration. In the presence of glucose, iodoacetate supressed the glucose-dependent increment in protein synthesis and caused a severe depletion of the intracellular ATP pool, probably as a result of accumulation of phosphorylated products which are proximal to the metabolic block. When pyruvate was added to an iodoacetate-blocked system, it was found to restore the incorporation of [^{3}H]leucine at a rate that equalled that measured in the absence of iodoacetate. Pyruvate abolished the effect of iodoacetate on [^{3}H]leucine incorporation both in glucose-containing and glucose-deficient media. The reversal of inhibition, effected by pyruvate, on [^{3}H]leucine incorporation was not the result of a repletion of the ATP pool and was specific to this metabolite. Neither lactate (Table 5) nor various intermediates of energy metabolism (Table 6) could replace pyruvate in the above respect.

Discussion

Glucose is known to have a role as a source of energy and of carbon and hydrogen atoms in biosynthetic pathways. In the present investigation

Table 5. *Effect of iodoacetate and of pyruvate on the glucose-induced stimulation of protein synthesis*

Cells were incubated for 30min at 37°C with the standard medium also containing 40μM-[^{3}H]leucine (1.1×10^4c.p.m./nmol) and glucose (5mM), pyruvate (5mM) or lactate (5mM) as indicated. For the analytical methods used see the Experimental section. Two representative experiments, conducted in two different batches of cells, are shown.

		[^{3}H]Leucine incorporation (nmol/mg of protein)		ATP (nmol/mg of protein)	
Expt.	Additions	No iodoacetate	Iodoacetate (0.1mM)	No iodoacetate	Iodoacetate (0.1mM)
(I)	None	3.3	2.6	18	19
	Glucose	5.1	2.6	24	5
	Pyruvate	3.0	3.3	18	15
	Lactate	3.3	2.5	17	16
	Glucose+pyruvate	4.7	5.0	17	5
	Glucose+lactate	4.8	2.6	18	5
(II)	None	2.4	2.0	23	20
	Glucose	3.2	1.7	24	4
	Pyruvate	2.8	2.4	23	28
	Glucose+pyruvate	3.2	3.3	23	8

Table 6. *Comparison between the effect of pyruvate and of some other metabolites on [^{3}H]leucine incorporation in the presence of iodoacetate*

Iodoacetate (0.1mM) and 5mM concentration of the respective metabolite were present as indicated. Conditions and the analytical methods used were as described in Table 5.

	[^{3}H]Leucine incorporation (nmol/mg of protein)		ATP (nmol/mg of protein)	
Additions	No glucose	Glucose (5mM)	No glucose	Glucose (5mM)
None	2.4	3.2	23	24
Iodoacetate	2.0	1.7	18	4
Iodoacetate+pyruvate	2.4	3.3	20	8
Iodoacetate+β-hydroxybutyrate	2.0	2.2	23	3
Iodoacetate+α-oxoglutarate	1.9	1.8	23	5
Iodoacetate+succinate	2.0	1.9	23	3

glucose was also identified as a factor in the regulation of protein synthesis in cultured rat heart muscle cells. A stimulatory effect of glucose on protein synthesis has previously been observed in various animal tissues (Hollinger & Hwang, 1972; Betteridge & Wallis, 1974; Permutt, 1974) and in cultured mammalian cells (Freudenberg & Mager, 1971; Griffiths, 1972; Van Venrooij *et al.*, 1972) but the effect was generally attributed to an increased ATP supply. An increased rate of ATP production is not the cause of the glucose-dependent stimulation of protein synthesis in cultured heart muscle cells. Several observations indicate that under both aerobic and anaerobic conditions, glucose stimulated the initial rate of protein synthesis without causing a concomitant change in the intracellular ATP concentration. In the absence of glucose, the anaerobic incorporation of [^{3}H]leucine into the cell proteins ceased before an appreciable change in the ATP concentration. Pyruvate restored the effect of glucose on protein synthesis in an iodoacetate-blocked system, again without a concurrent change in the ATP concentration.

In the light of the above findings it seems more likely that glucose or one of its metabolic products accelerates the limiting step in the sequence of reactions that lead to the incorporation of a labelled amino acid into the polypeptide chain. In the case of an extracellular amino acid, this sequence also includes the process of transport. Although one cannot generalize from the study of a single amino acid analogue, glucose did not stimulate α-aminoisobutyric acid transport. There is also no indication that the glucose effect would be mediated by an inhibition of protein degradation (see Table 3). In this context it is noteworthy that Fulks *et al.* (1975) reported a glucose-induced stimulation of protein synthesis in rat diaphragms and attributed the latter mainly to an inhibition of protein degradation. Thus the cultured heart muscle cells seem to differ in this respect from the diaphragm. It is also unlikely that the glucose effect on protein synthesis is mediated through the

stimulation of mRNA formation. The effect of glucose on the incorporation of [^{3}H]leucine was already noticeable 5min after its addition. This time is too short to allow for the movement of the mRNA from the nucleus into the cytoplasm, the site of initiation and elongation of the polypeptide chain (cf. Penman *et al.*, 1968). That the glucose-dependent increment is not sensitive to actinomycin D is also in accord with the suggestion that the glucose effect is not on the transcriptional level. The plausible sites for the glucose effect are, therefore, initiation and elongation. There is as yet no direct evidence for this contention but some support in favour of this view can be obtained from the literature. Freudenberg & Mager (1971) and Van Venrooij *et al.* (1972) found that anaerobic incubation of rabbit reticulocytes or Ehrlich ascites cells respectively in the absence of glucose stopped, in an early stage, the process of elongation and, in a later stage, also initiation.

Iodoacetate was found to abolish the glucose stimulation of protein synthesis. In the presence of this inhibitor of glycolysis, pyruvate by itself had only a slight influence on [^{3}H]leucine incorporation. Therefore it is rather surprising that the concerted action of glucose and pyruvate on protein synthesis was not suppressed by iodoacetate. Interestingly, a strikingly similar observation has been reported by Matschinsky & Ellerman (1973). These authors found that the capacity of glucose to release insulin from pancreatic islets was blocked by iodoacetate, but that pyruvate relieved the inhibitory effect of iodoacetate on the glucose-mediated insulin release. Pyruvate by itself does not induce hormone secretion.

One possible interpretation of the effect of pyruvate on heart muscle cells might be that the stimulation of protein synthesis requires the simultaneous action of two substances. One of these two substances must be an intermediate formed from glucose in a metabolic step before that blocked by iodoacetate (i.e. glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate or triose phosphate). The other should be either pyruvate or a substance derived from it. An alternative is that only one of the intermediates formed from glucose in the presence of iodoacetate is necessary for the stimulation of protein synthesis. Pyruvate might then exert its effect by influencing one of the enzymic steps on the glucose side of the iodoacetate block and thereby change the relative ratio of the phosphorylated products that accumulate in the cell.

At present, the suggestion that the glucose-dependent stimulation of protein synthesis is related to the known role of glucose in the prevention of irreversible damage to the anoxic myocardium, is still purely speculative. It has yet to be established whether glucose stimulates protein synthesis non-specifically or whether its effect is restricted to specific classes of proteins. Moreover, it will also be of interest to find out whether glucose-stimulated protein synthesis, which is not mediated by ATP generation, occurs only in cultured heart cells or is of more general occurrence.

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