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Inhibition of glucose phosphorylation by fatty acids in the perfused rat heart

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The flux of glucose entering the glycolytic pathway under various metabolic conditions has been indirectly monitored in the Langendorff perfused rat heart using ^{31}P-NMR spectroscopy. By totally inhibiting (>95%) glyceraldehyde-3-phosphate dehydrogenase with low concentrations of iodoacetic acid (0.2 mM) in the perfusion medium, active glycolysis results in the accumulation of sugar phosphate species (fructose 1,6-bisphosphate, dihydroxyacetone phosphate, and glyceraldehyde 3-phosphate) which can be observed in the ^{31}P-NMR spectrum. Using this technique, it has been shown that butyrate (10 mM) in the perfusion medium decreases the flux through the initial steps of the glycolytic pathway by at least 6-fold and that both glucose phosphorylation and glycogenolysis are inhibited. Upon total global ischemia in the presence of both glucose and butyrate, the glycolysis rate is stimulated approx. 100-fold.

^{31}P-NMR; Glycolysis; Fatty acid; Iodoacetate; Glyceraldehyde-3-phosphate dehydrogenase

1. INTRODUCTION

The availability of fatty acids as an alternative energy source for the glucose-perfused rat heart has been shown to inhibit significantly the utilization of glucose [1–4]. Although the exact mechanism by which the presence of fatty acids inhibits glucose utilization is somewhat uncertain, the observed accumulation of glucose [3] in the heart when both glucose and fatty acids are provided in the medium suggests that the inhibition of glucose utilization occurs after glucose uptake but before the first phosphorylation event. ^{31}P-NMR spectroscopy of the Langendorff perfused rat heart can be used to follow changes in the concentrations of major pools of phosphorus-containing metabolites in vivo [5]. However, the normally low steady-state concentrations of the phosphorus-containing intermediates of glycolysis generally preclude their direct detection by ^{31}P-NMR spectroscopy. By totally (>95%) inhibiting glycolysis at the level of glyceraldehyde-3-phosphate dehydrogenase by perfusion with iodoacetic acid (0.2 mM) [6], it is possible to observe the flux through the initial steps of glycolysis as an accumulation of an equilibrium mixture of fructose 1,6-bisphosphate, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (Chatham, J.C., Gilbert, H.F. and Radda, G.K., unpublished) which gives rise to signals in the ^{31}P-NMR spectrum. Using this technique it has been shown that the presence of butyrate in the perfusion medium significantly inhibits glycolysis and glycogenolysis and that upon total global ischemia, glycolysis is greatly stimulated without a significant alteration in intracellular pH because lactate production is inhibited under these conditions.

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2. MATERIALS AND METHODS

Hearts were excised from ether-anesthetized male Wistar rats and perfused at 37°C in the Langendorff mode with phosphate-free Krebs-Henseleit-bicarbonate buffer as described [7]. The

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buffer was continually equilibrated with 95% O_2/5% CO_2 to maintain a pH of 7.4. All chemicals were of analytical grade. Sodium butyrate when present was added at 10 mM, and the NaCl concentration decreased to maintain constant sodium concentration. Glucose when present was at 11 mM. Iodoacetic acid (sodium salt) adjusted to pH 7.4 (10 mM) was added to the perfusion line by a peristaltic pump with the flow rate adjusted so that the final concentration of iodoacetate in the perfusate was 0.2 mM.

The activity of glyceraldehyde-3-phosphate dehydrogenase was assayed according to [8] after homogenizing heart tissue in 9 vols triethanolamine buffer (0.1 M, pH 7.5) containing 1 mM EDTA and 5 mM $MgSO_4$. ^{31}P-NMR spectra were collected on hearts perfused as described above by using a 4.2 T vertical wide-bore superconducting magnet interfaced with a Nicolet 1180 spectrometer. Spectra were collected in 5-min blocks (256 scans, 70° pulse width). Peak intensities were determined relative to the signal from a methylene diphosphonate (MDP) standard contained in a capillary in the sample chamber. Calibration of the total sugar phosphate concentration in individual perfused hearts was accomplished by relating the heights of the observed sugar phosphate peak (relative to the height of the internal MDP standard) to the level of fructose 1,6-bisphosphate plus dihydroxyacetone phosphate and glyceraldehyde 3-phosphate determined by enzymatic methods in acid extracts of heart homogenates. Fructose 1,6-bisphosphate in neutralized acid extracts of heart tissue was determined by measuring the decrease in absorbance at 340 nm due to NADH oxidation in the presence of 5 U/ml of aldolase and glycerol-3-phosphate dehydrogenase. The sum of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate was evaluated by observing the decrease in absorbance at 340 nm due to NADH oxidation in the presence of 5 U/ml triosephosphate isomerase and glycerol-3-phosphate dehydrogenase. One arbitrary unit of sugar phosphate peak height relative to the external MDP standard shown in fig.2 corresponds to a concentration of fructose 1,6-bisphosphate plus triose phosphates of 3.7 ± 0.3 μmol/g tissue per peak height unit (average of duplicate determinations). Glucose 6-phosphate plus fructose 6-phosphate represented less than 20% of the sugar phosphate pool.

3. RESULTS AND DISCUSSION

^{31}P-NMR spectroscopy of intact tissue has been used to monitor the changes in major phosphorus-containing metabolites, to determine the intracellular pH, and to estimate the concentration of free ADP under a variety of metabolic conditions [5]. The utility of ^{31}P-NMR for the in vivo determination of the concentrations of phosphorus metabolites is restricted by the inherent insensitivity of the technique. Generally, only the most abundant phosphorus-containing metabolites are observed. In the normal perfused rat heart, ^{31}P-NMR signals are observed for only ATP, phosphocreatine and inorganic phosphate. Interconversions of these abundant metabolites can be examined in vivo in a non-destructive, non-invasive manner. However, it is generally impossible to observe the presence of a very large number of phosphorus metabolites of lower concentration (<1 mM). ADP concentrations can be inferred from the equilibrium position of creatine kinase, but the phosphorus-containing intermediates of glycolysis cannot normally be detected. In the presence of a specific inhibitor, however, the normally invisible intermediates of glycolysis will accumulate behind the inhibited step. The accumulation of these normally invisible intermediates can then be used to estimate the flux through glycolysis. Thus, metabolic inhibitors can be used to extend the utility of ^{31}P-NMR methods.

The addition of 0.2 mM iodoacetate to normal Krebs-Henseleit buffer containing 11 mM glucose results in a very rapid disappearance of ATP and phosphocreatine signals with the concurrent appearance of new resonances in the sugar phosphate region of the spectrum (figs 1,2) and irreversible loss of cardiac function. Assay of phosphorus-containing metabolites from homogenates of rapidly frozen tissue after perfusion with iodoacetate showed a significant increase in the levels of fructose 1,6-bisphosphate, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (not shown), similar to the increase observed upon inhibition of glyceraldehyde-3-phosphate dehydrogenase by perfusion with hydrogen peroxide (Chatham, J.C., Gilbert, H.F. and Radda, G.K., unpublished). Assays of enzyme activity showed that hexokinase, phosphofructokinase and pyruvate kinase activities of the iodoacetate-treated hearts were normal but that the activity of glyceraldehyde-3-phosphate dehydrogenase was >95% inhibited (not shown).

The rate of accumulation of sugar phosphate resonances (fig.2) is comparable to the expected rate of glycolysis (table 1); however, the rate of sugar phosphate accumulation after adding iodoacetate to the perfusion may also reflect the rate of inactivation of glyceraldehyde-phosphate dehydrogenase, rather than providing a true measure of the actual rate of glycolysis during the initial perfusion.

The inclusion of butyrate, an alternative substrate for oxidative phosphorylation which does not rely on glycolysis for metabolism, protects

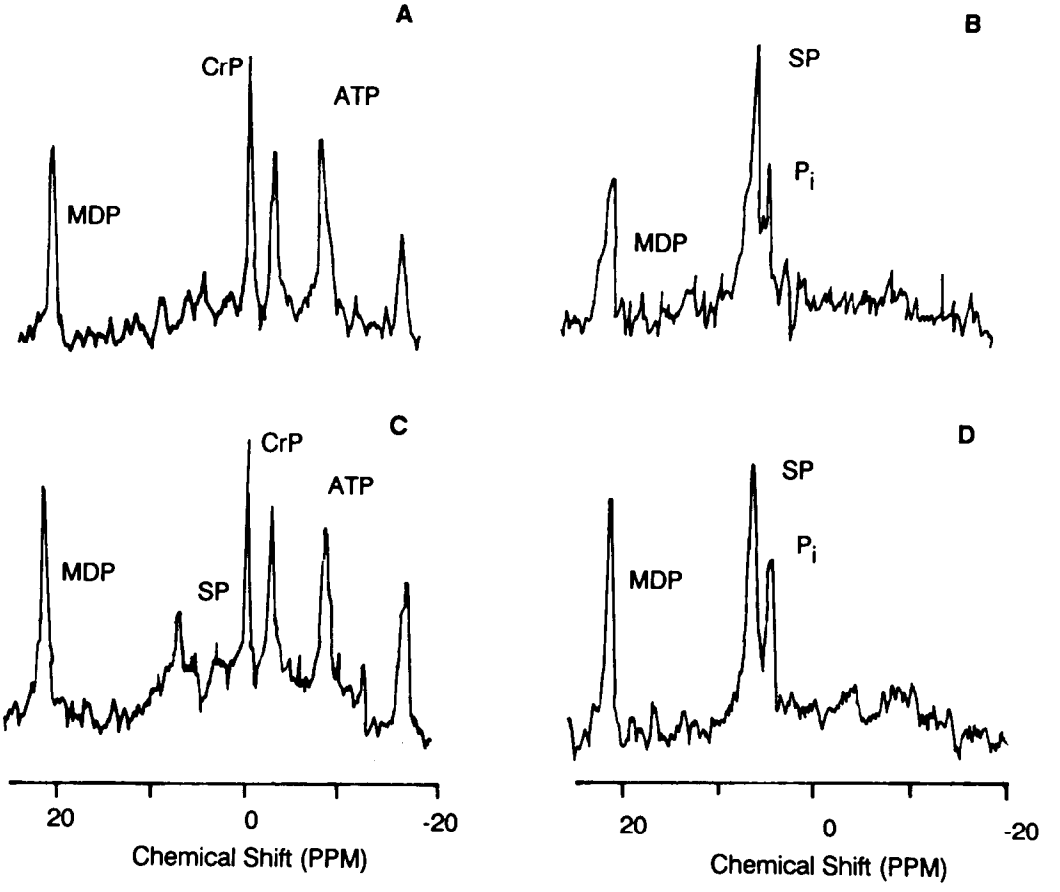

Fig.1. Typical ^{31}P-NMR spectra of the Langendorff perfused rat heart under various conditions. (A) Control heart perfused for 30 min with 11 mM glucose in the absence of iodoacetate or butyrate. (B) Heart perfused for 30 min with 11 mM glucose and 0.2 mM iodoacetate. (C) Heart perfused for 35 min with 11 mM glucose, 0.2 mM iodoacetate, and 10 mM butyrate. (D) Heart perfused for 20 min as in (C) and then subjected to 10 min of total global ischemia. Peak identification: MDP, methylene diphoshonate; SP, sugar phosphates; P_i, inorganic phosphate; CrP, creatine phosphate; ATP, α-, β- and γ-phosphates.

against the effects of iodoacetate with only small losses in ATP or phosphocreatine and no decrease in function for up to 1 h (figs 1,2). Assays of glyceraldehyde-3-phosphate dehydrogenase activity in heart homogenates perfused with butyrate, glucose, and iodoacetate show $< 5\%$ of the normal activity of the enzyme. Although the enzyme is completely inhibited, there is no significant accumulation of sugar phosphate in the heart when butyrate is present (fig.2, table 1). Phosphorylation of glucose can occur in both the presence and absence of iodoacetate; however, in the presence of iodoacetate, a significant flux through the initial stages of glycolysis would result in the accumulation of fructose 1,6-bisphosphate and triose phosphates, since glyceraldehyde-3-phosphate dehydrogenase is inhibited. In the presence of iodoacetate, the sugar phosphates which accumulate are predominately ($>80\%$) fructose 1,6-bisphosphate and the triose phosphates. In the presence of iodoacetate and butyrate, the slow appearance of sugar phosphate signals in the NMR spectrum suggests that butyrate in the perfusion medium produces at least a 6-fold reduction in the rate of glycolysis. If the inhibition of glycolysis by butyrate occurred after the initial phosphorylation of glucose by hexokinase but before the glyceraldehyde-3-phosphate dehydrogenase step, other sugar phosphates (such as glucose 6-phosphate, glucose 1-phosphate, or fructose 6-phosphate)

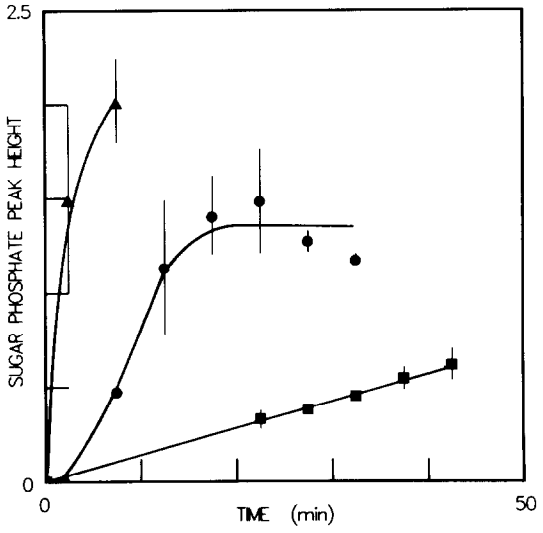

Fig.2. Time courses of the accumulation of sugar phosphate resonances. (●) Perfusion with 11 mM glucose and 0.2 mM iodoacetate; (■) perfusion with 11 mM glucose, 0.2 mM iodoacetate and 10 mM butyrate; (▲) perfusion with 11 mM glucose, 0.2 mM iodoacetate and 10 mM butyrate for 20 min followed by total global ischemia at time zero. All peak heights have been normalized to the MDP standard and are the average of at least three individual experiments. Error bars indicate ± 1 SD.

would be expected to accumulate at the normal rate. Since this is not observed, the inhibition of glycolysis by butyrate likely occurs before phosphorylation of glucose. These observations are in agreement with previous studies showing a decrease in the rate of glycolysis [1,2,4] and accumulation of free intracellular glucose [3] in the presence of butyrate. The results also suggest that the effects of iodoacetate on high-energy phosphate metabolism are reasonably specific.

If hearts perfused with iodoacetate, glucose and butyrate are subjected to a period of total global ischemia, there is a very rapid accumulation of sugar phosphate and a correspondingly rapid loss of function (figs 1,2). The rate of sugar phosphate accumulation upon total global ischemia is accelerated 100-fold compared to the normoxic rate in the presence of butyrate (table 1), and approaches a rate comparable to the rate of glycolysis during anoxia as measured by others (table 1).

The rapid accumulation of sugar phosphate as a consequence of total global ischemia is not accompanied by a significant change in intracellular pH.

Table 1

Rates of glycolysis in the Langendorff perfused rat heart under various metabolic conditions

Additions	Glycolysis rate	Reference
No additions		
11.5 mM glucose	0.54	4
5.5 mM glucose + insulin (1 mU/ml)	1.3	2
5.5 mM glucose + insulin + 4 mM butyrate	0.66	2
10 mM glucose + anoxia	4.7	9
Iodoacetate (0.2 mM)		
11.5 mM glucose	0.34 ± 0.07	this study
11.5 mM glucose + 4 mM butyrate	0.05 ± 0.005	this study
11.5 mM glucose + 4 mM butyrate + total global ischemia	≥6	this study

All hearts were perfused at 37°C with Krebs-Henseleit buffer containing the additions listed. Glycolysis rates are reported in units of μmol glucose/min per g wet wt heart tissue. Rate measurements are based on the curves shown in fig.2. Errors given encompass the range of values consistent with error in the individual time points

pH values measured by NMR [5] were 7.03 and 6.91 before and after ischemia, respectively. The lack of a significant pH drop in response to significant acceleration of glycolysis is also consistent with the inhibition of lactate formation caused by inhibition of glycolysis at the glyceraldehyde-3-phosphate dehydrogenase step.

In summary, ^{31}P-NMR of the perfused rat heart has confirmed the inhibition of glucose phosphorylation in vivo by the presence of fatty acids in the perfusion medium. The acceleration of glycolysis during ischemia has also been shown to occur in the absence of a significant change in intracellular pH. The use of specific metabolic inhibitors provides a useful supplement to ^{31}P-NMR studies of metabolism in vivo.

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