Rates of Glycolysis and Glycogenolysis During Ischemia in Glucose-Insulin-Potassium–Treated Perfused Hearts: A \(^{13}\)C, \(^{31}\)P Nuclear Magnetic Resonance Study

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The effects of 11.7 mM glucose, insulin, and potassium (GIK) on metabolism during ischemia were investigated in the perfused guinea pig heart using magnetic resonance spectroscopy. Intracellular metabolites, primarily glycogen and glutamate, were labeled with \(^{13}\)C by addition of [1-\(^{13}\)C]glucose to the perfusate during a normoxic, preischemic period. \(^{13}\)C and \(^{31}\)P NMR spectroscopy was used to observe the metabolism of \(^{13}\)C-labeled metabolites simultaneously with high-energy phosphorus metabolites and pH. The extent of acidosis and the rate and amount of labeled lactate accumulation during ischemia were the same in control (3 mM glucose + insulin) and GIK-treated hearts. In contrast, the rate of labeled glycogen mobilization during ischemia in GIK-treated hearts was one third the rate observed in control hearts. These observations suggest that GIK decreased the rate of glycolysis during ischemia without affecting the rate of glycolysis. We propose that glucose contributed as a glycolytic substrate to a greater extent during ischemia in GIK-treated hearts than in hearts perfused with 3 mM glucose and insulin. The glycogen-sparing effect of GIK demonstrated in these studies could delay the onset of ischemic damage in a clinical setting by prolonging the availability of glycolytic substrate necessary for production of high-energy phosphate. (Circulation Research 1988;62:1065-1074)

Myocardial ischemia is a common and important clinical problem. Although metabolism of the ischemic myocardium has been an area of active investigation for many years, the metabolic changes resulting from the inadequate oxygen and substrate supply and product removal associated with ischemia are incompletely understood. However, it is clear that one of the important metabolic events leading to cellular damage during ischemia is failure of the rate of energy production to meet the demands for energy utilization.

Several therapeutic strategies have been introduced to alter cardiac metabolism during ischemia. A mixture of glucose, insulin, and potassium (GIK) was initially used 25 years ago as a means of eliminating the electrocardiographic abnormalities of myocardial infarction. Since that time GIK has been used in numerous studies of coronary artery occlusion in animals and man. Nearly all of the animal studies, using a variety of measures of recovery, have shown reduced infarct size and improved tissue metabolism. Studies in man have been less conclusive. Infusion of GIK in patients with acute myocardial infarction has resulted in improved left ventricular function, improved electrical stability, clinical improvement, and reduced mortality. Other studies have found no reduction in mortality. In patients with stable angina pectoris and coronary artery disease, administration of GIK, together with cardiac pacing, was beneficial in some studies and detrimental in others. A better understanding of the metabolic action of GIK may explain many of these contradictory results. Several mechanisms proposed for the action of GIK are 1) to increase anaerobic and/or aerobic glycolysis, 2) to spare glycogen, 3) to reduce "toxic" levels of free fatty acids and their consequent detrimental effects, 4) to stabilize membranes, and 5) to replete intracellular potassium. Nuclear magnetic resonance (NMR) spectroscopy is ideally suited to examine noninvasively the effects of GIK on glycogenolysis and glycolysis during ischemia; therefore, we address the first and second mechanisms in this study.

\(^{31}\)P NMR spectroscopy has been used extensively to study the metabolism of high energy phosphorus metabolites and intracellular pH in isolated hearts and in vivo. However, pathways of carbon metabolism are centrally involved in the production of energy, and the ability to observe these by \(^{13}\)C NMR greatly extends the power of NMR to study metabolism. We report here the use of \(^{13}\)C and \(^{31}\)P NMR spectroscopy to correlate in time the metabolism of labeled carbon metabolites with changes in high energy phosphorus metabolites and pH during ischemia and reflow in the isolated Langendorff-perfused guinea pig heart. \(^{13}\)C and \(^{31}\)P spectra were collected on an alternate scan...
basis, allowing us to observe metabolites containing phosphorus and carbon simultaneously on each heart. This combination is much more powerful than observation of either nucleus alone.

The focus of this study is the effect of GIK on rates of glycogenolysis and glycolysis during ischemia. GIK-treated hearts were compared with hearts perfused with 3 mM glucose (fasting level) and insulin. Intracellular metabolites, primarily glycogen and glutamate, were labeled from exogenously supplied [1-13C]glucose during a preischemic aerobic perfusion period. Glycogenolysis of the [1-13C]glucose was subsequently monitored by 13C NMR spectroscopy during ischemia as it was utilized in the glycolytic pathway to form [3-13C]lactate and other products. The extent and rate of glycolysis were estimated from the labeled lactate formed. High-energy phosphorus metabolites were monitored by 31P NMR spectroscopy, and intracellular acidosis was determined by calculation of pH from the chemical shift of the internal inorganic phosphate (Pi) resonance.

**Materials and Methods**

**Chemicals**

[1-13C]Glucose, 99 atom percent, was synthesized by published procedures. Glucose enzymatic assay kit and insulin (bovine) were obtained from Sigma Chemical, St. Louis, Missouri. All other chemicals used were analytical grade or better.

**Animals**

Male Hartley strain guinea pigs (Charles River Breeding Labs, Wilmington, Massachusetts) weighing 400–700 g were used in all experiments. They were fed ad libitum (Waynes Mills nonmedicated guinea pig feed).

**Perfusate**

The perfusate (200 ml) was Krebs-Henseleit bicarbonate buffer prepared fresh for each experiment, containing 0.1 ml/ml streptomycin, 100 units/ml penicillin, and 25 mg/ml amphotericin B. Labeled substrate used was [1-13C]glucose (3.0 mM or 11.7 mM). Where appropriate, glucose-insulin-potassium was added to the perfusate to give concentrations of 11.7 mM, 2.5 units/l, and 0.55 mM, respectively. In some experiments, 11.7 mM [1-13C]glucose was used as part of the GIK solution.

**Perfusion Apparatus and Measurements**

Hearts were perfused by the Langendorff method within the magnet using a home-built apparatus consisting of a perfusate reservoir, peristaltic pump, filter, membrane oxygenator (Sci Med, Minneapolis, Minnesota), and bubble trap. Perfusion pressure was regulated by an overflow valve in the line delivering perfusate to the aorta. Functional viability of the hearts during the NMR experiments was monitored by a Statham transducer located in the perfusion line to the aorta. Heart rates and rate pressure product (heart rate x relative aortic pressure) were constant during the normoxic labeling period. Upon reflow the heart rates and rate pressure products were 10% and 11% greater, respectively, than before ischemia, demonstrating viability of the hearts. This was true for GIK-treated as well as control (3 mM glucose) hearts. No significant difference was found between GIK-treated and control hearts with respect to heart rate increase or increase in rate pressure product after ischemia. A similar increase in heart rate after ischemia has been seen by others.

Absolute indexes of left ventricular function, measured in hearts that had a latex balloon inserted through the mitral valve into the left ventricle, could not be measured in the magnet because signals from the balloon interfered with signals from 13C metabolites. These measurements were made on hearts, perfused outside of the magnet, under conditions identical to the normoxic period during the NMR experiments. Left ventricular developed pressure (LVDP) for these hearts was 108 mm Hg with an end-diastolic pressure (EDP) set at 5 mm Hg; dp/dt max was 2,910 mm Hg/sec; double product was 21,820 mm Hg/min. The perfusate was maintained at 37°C by thermostated water jackets on the individual components of the apparatus. Gases for oxygenation were delivered to the membrane oxygenator through a humidifier at a rate of 1,200 ml/min (95% O2-5% CO2). The pH of the perfusate was maintained between 7.35 and 7.45 by minor adjustments of the CO2 flow rate. Oxygen uptake of the heart was monitored with a Clark electrode and dissolved oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio). Oxygen concentration in perfusate delivered to the heart was 24.9 ppm. Uptake was 18.2 μmol/min/g dry wt, which was 56% utilization of the available oxygen. The presence of a latex balloon in the left ventricle did not affect oxygen uptake. In our Langendorff perfusion, the heart is submerged in perfusate; presumably, the left ventricle can pump some fluid. For total global ischemia, the inlet to the aorta was clamped.

**Preparation of Isolated Perfused Hearts**

Guinea pigs were anesthetized with sodium pentobarbital (75 mg/kg i.p.); hearts were rapidly removed and dropped into iced saline. Any lung tissue excised with the heart was removed; a polymer cannula was sutured into the aorta. Retrograde perfusion was immediately begun with Krebs-Henseleit buffer at 37°C and 90 cm water pressure. After 5 minutes of perfusion without recirculation of perfusate, the heart was placed in a 20-mm diameter NMR tube sealed with a Teflon plug containing inlet and exit tubings. At this time, the perfusate was recirculated and the heart was positioned in the magnet. After a few minutes for probe tuning and field homogeneity adjustment, data accumulation was initiated. Coronary flow was 12–20 ml/min for hearts weighing 1.7–2.0 g wet. Hearts having PCr/ATP less than 3 or having inadequate function, as judged from low heart rates or low relative aortic pressure, were rejected.7

**NMR Measurements**

High-resolution NMR spectra were obtained at 7.0 T using a dual-tuned NMR probe constructed by Cryo-
magnet systems (Indianapolis, Indiana) and two spectrometer consoles. A Bruker WM300 console provided proton decoupling at 300 MHz, $^{13}$C excitation and detection at 121 MHz, and triggered the second console, an AM200, to perform $^{13}$C excitation and detection. By using two consoles, we could obtain spectra of two nuclei on an alternate single scan basis. Consequently, one nucleus was detected while the other nucleus was relaxing. $^{13}$C spectra were broad band proton-decoupled in the gated two-level mode, using 8 W $^1$H irradiation during $^{13}$C acquisition and 1.5 W during the delay. The temperature of hearts in the magnet was monitored by a fluoroptic thermometer (Luxtron Corp, Mountain View, California) and controlled by the Bruker temperature controller accessory and by the delivery of temperature-controlled perfusate to the heart. $^{13}$C spectra were recorded using 45° excitation pulses, 17,000 Hz spectral width, 8K data points, and 0.25-second acquisition time. $^3$P spectra were obtained using 45° pulses, 9,800 Hz spectral width, and 0.42-second acquisition time. All spectra shown are transforms of 256 acquisitions, with a 1.2-second pulse interval (4.96 minutes total acquisition time). For some experiments, better kinetic data were obtained using spectra recorded with 192 or 128 scans. Lorentzian lineshape analysis of transforms from this number of acquisitions gave accurate measurements of integrated intensities. Magnetic field homogeneity was optimized by shimming on the proton resonance from water. $^{13}$C and $^3$P chemical shifts are reported, using the IUPAC convention, relative to tetramethylsilane at 0 ppm, using $^1$H glucose at 97 ppm, and phosphocreatine (PCr) at 0 ppm, respectively, as internal reference standards.

**Experimental Protocol**

To correlate in time the metabolism of labeled carbon and phosphorus metabolites, [1-$^{13}$C]glucose was added to the perfusate at the beginning of a 40-minute preischemic, normoxic period to label intracellular metabolites. $^{13}$C and $^3$P NMR spectra were collected throughout the preischemic (40-minute), ischemic (15-minute), and reflow (50-minute) periods. To examine the effects of GIK on myocardial metabolism, hearts were initially perfused for 40 minutes, under normal perfusate flow rates, with perfusate containing either 11.7 mM [1-$^{13}$C]glucose, insulin, and potassium (GIK-treated), or 3 mM [1-$^{13}$C]glucose and insulin (control). Insulin was included in both sets of experiments to minimize the variables being tested and ensure maximal glucose uptake during the labeling period. Hearts were then subjected to total global ischemia for 15 minutes, followed by a return to normal perfusate flow rates for an additional 50 minutes. In some experiments, GIK was added only at reflow.

**NMR Data Analysis**

A capillary tube containing $^{13}$C formate and GdCl$_2$ was mounted on the inner wall of the NMR sample tube to monitor changes in spectrometer and probe sensitivity and to serve as an intensity reference standard. This intensity standard was calibrated by comparison with known concentrations of glucose contained in the sample buffer. For some experiments, however, the resonance from the formate in the capillary was extremely broad, presumably because of poor $^1$H homogeneity in the localized region of the capillary. This created difficulties in determining its intensity, even when Lorentzian line-shape analysis was used in determining the integrated intensity. Consequently, the $\alpha$- and $\beta$-1$^3$C resonances from the infused glucose at the start of the experiment were used as a secondary intensity reference, with proper allowances made for the concentration differences between experiments. In experiments where the two reference methods could be compared, they yielded equivalent results (within 10%). For consistency in all experiments, the reported intensities are all derived from the secondary reference.

Integrated intensities for the $^{13}$C resonances from glycogen, glucose, lactate, and alanine, and $^3$P resonances from inorganic phosphate, PCr, and $\beta$-ATP were determined by Lorentzian line-shape analysis (fit to sum of Lorentzian lines) using NMRI software supplied by the National Institutes of Health resource for NMR data analysis (Syracuse, New York) on a VAX 11/780 computer. $^{13}$C spin-lattice relaxation rates were determined for glucose and glycogen (in normoxic hearts) and lactate (in hypoxic hearts), and the measured integrals for glucose, glycogen, and lactate were corrected for $T_1$ (spin-lattice relaxation time) saturation effects. An important assumption in applying these corrections is that the $T_1$s measured under normoxic and hypoxic conditions are reasonable estimates of the relaxation times of metabolites in ischemic tissue. We emphasize that the saturation correction factors for glucose and glycogen do not show a strong dependence on $T_1$; doubling the $T_1$ of glycogen, for example, changes the saturation factor by only 3%. Consequently, major changes in metabolite $T_1$s would have to occur during the experiment to significantly change the degree of saturation. The reported absolute lactate accumulation rates are subject to the greatest uncertainty, since the integrals have a much larger correction and the intracellular or extracellular location of lactate is the least well known of the metabolites reported. However, the errors introduced by these assumptions and the corrections for nuclear Overhauser effects (NOE), described below, are systematic and should apply equally to both GIK-treated and control hearts.

In vitro NOEs were measured for labeled glycogen isolated from hearts and for glucose and lactate in phosphate buffered saline (150 mM NaCl, 10 mM Na$_2$HPO$_4$, pH 7.4) containing 3 mM MgCl$_2$. In correcting for NOE effects, we have assumed that the NOEs are similar in the perfused heart and in vitro. This was shown to be true for liver glycogen. The resonances from glucose in particular, and lactate, at least in part, are derived from extracellular metabolites (see below), so the in vitro measurements of their NOE...
should be a reasonable estimate of the NOE in a perfusion experiment.

The rates of labeled glycogen synthesis and mobilization and lactate accumulation were determined from plots of integrals corrected for $T_1$ saturation and NOE effects versus time by estimating the slope of the initial part of the curve and converting to micromoles $^{13}$C per minute per gram dry weight. The amount of labeled lactate accumulated after 15 minutes of ischemia was determined from the integrated intensities at the end of the ischemic period. The pH values reported were calculated from the chemical shift of the internal inorganic phosphate resonance using the following equation:

$$pH = pK_a - \log(\delta - \delta_0 / \delta_a - \delta)$$

$pK_a = 6.79$; $\delta_a = 5.75$; $\delta_0 = 3.25$. Intracellular pH calculated from $^{31}$P NMR data is accurate to 0.1 pH units and changes accurate to 0.05 pH units.

**Chemical Analyses**

Some hearts were freeze-clamped with Wollenberger tongs, chilled in liquid nitrogen, for subsequent extraction. Hearts were frozen either before perfusion, at the end of 40 minutes of normoxic perfusion, after 15 minutes of subsequent ischemia, or after 50 minutes of reflow after the ischemic period. Heart tissue was powdered at liquid nitrogen temperature and fractions taken for wet/dry determination, perchloric acid extraction, or alkaline extraction. Neutralized perchloric acid extracts were used for assay of glucose (enzymatic assay, Sigma) and amino acids (model 7300 amino acid analyzer, Beckman, Fullerton, California). Alkaline extraction, hydrolysis, and assay of glycogen were carried out by minor modifications of published methods.

**Results**

**Aerobic Incorporation of $^{13}$C Label**

Proton decoupled $^{13}$C spectra from a heart perfused with 3 mM [1-$^{13}$C]glucose and insulin (control) are shown in Figure 1A. In the carbon spectra, the resonances at 97 and 93 ppm are from $\beta$- and $\alpha$-[1-$^{13}$C]glucose, respectively. Within 2–7 minutes after the precursor reached the heart, labeled carbon was detected in C1 of glycogen and C4 of glutamate. Somewhat later, label appeared in C2 and C3 of glutamate, and to a lesser extent, in C2 and C3 of aspartate. The weak resonance at 30 ppm is from naturally abundant $^{13}$C in the methylene groups of fatty acids of triglycerides; the naturally abundant fatty acyl olefinic groups appear at 130 ppm (not shown). The $^{31}$P spectra from the same heart, recorded during the same periods after beginning the perfusion, are shown in Figure 1B. The assignments of the resonances are the same as those reported in the literature. Incorporation of label into glycogen was linear for the duration of the preischemic period (40–50 minutes), in contrast to the plateau of glutamate resonance intensities within 7–10 minutes after addition of label. This difference is most simply explained by assuming that labeling of the glycogen pool occurs by net synthesis.

**$^{13}$C and $^{31}$P Spectra During Ischemia**

The changes in the $^{13}$C and $^{31}$P NMR spectra during 15 minutes of total global ischemia are shown in Figures 2A and 2B. The carbon spectra show a decrease in the intensity of the C1 glycolgen resonance and an accumulation of C3-labeled lactate, alanine, and to a...
FIGURE 1. A: $^{13}$C NMR spectra of an isolated guinea pig heart perfused with insulin and 3 mM $[1-^{13}C]$glucose. B: $^{31}$P NMR spectra, taken simultaneously with the $^{13}$C spectra, of the same heart. Accumulation times given at the right of the $^{31}$P spectra are relative to the beginning of the perfusion. Details are given in "Materials and Methods." Peak assignments are $\beta$-NTP, $\beta$-phosphate of nucleoside triphosphates (NTP), mostly adenosine triphosphate (ATP); $\alpha$, $\alpha$-phosphate of nucleoside triphosphates; $\gamma$, $\gamma$-phosphate of ATP; PCr, phosphocreatine; Pi, inorganic phosphate; PME, phosphomonoesters; GLUT C3, $[3-^{13}C]$glutamate; GLUT C4, $[4-^{13}C]$glutamate; ASP C3, $[3-^{13}C]$aspartate; ASP C2, $[2-^{13}C]$aspartate; GLUTC2, $[2-^{13}C]$glutamate; Gluc C1, $[1-^{13}C]$glucose; $\alpha$ Gluc, $\alpha$-[1-^{13}C]glucose; $\beta$ Gluc, $\beta$-[1-^{13}C]glucose; Gluc C1, $[1-^{13}C]$glycogen. $\alpha$-NTP and $\gamma$-ATP peaks may have minor contributions from $\alpha$-phosphate of nucleoside diphosphates and $\beta$-phosphate of ADP, respectively.

TABLE 1. $^{13}$C Heart Metabolites and pH During Normoxia, Ischemia, and Reflow

<table>
<thead>
<tr>
<th>Perfusate glucose</th>
<th>Glycogen synthesis rate*</th>
<th>Glycogen mobilization rate*</th>
<th>Lactate accumulation</th>
<th>Acidosis</th>
<th>Reflow</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 mM + insulin</td>
<td>2.90 (0.66)</td>
<td>6.11 (0.59)</td>
<td>8.88 (2.48)</td>
<td>69 (17)</td>
<td>7.08 (0.01)</td>
</tr>
<tr>
<td>n = 4</td>
<td></td>
<td>n = 4</td>
<td>Amount†</td>
<td>6.48 (0.08)</td>
<td>3.04 (0.07)</td>
</tr>
<tr>
<td>11.7 mM + insulin + K⁺</td>
<td>3.17 (0.36)§</td>
<td>2.34 (0.53)‡</td>
<td>9.01 (2.15)§</td>
<td>85 (13)†</td>
<td>7.09 (0.01)</td>
</tr>
<tr>
<td>n = 5</td>
<td></td>
<td>n = 4</td>
<td>Amount†</td>
<td>6.35 (0.05)§</td>
<td>2.94 (0.53)§</td>
</tr>
</tbody>
</table>

* Rates are expressed in $\mu$mol $^{13}$C/min/g dry wt as mean (standard deviation).
† Rates and amounts were corrected for $T_1$ (spin-lattice relaxation time) saturation and nuclear Overhauser effects.
‡ Significantly different from control, confidence interval 99.8%, Student’s t test.
§ Not significantly different from control values.
lesser extent, labeled glycerol 3-phosphate and succinate. The succinate resonance appears as a downfield shoulder on the more intense C4 glutamate resonance (inset). The changes in the 31P NMR spectra are similar to those reported previously.28,29 The PCr resonance decreased rapidly after the onset of ischemia, while the internal Pi resonance increased and shifted upfield, reflecting acidosis. The β-ATP resonance decreased by 20–25% during 15 minutes of ischemia. The decreases in β-ATP and PCr were not significantly different between the control and GIK-treated hearts. Carbon spectra for GIK-treated hearts were similar to these for the control hearts with the exception of glycogen mobilization rate, discussed below. Accurate quantitation of glucose utilization from these spectra was not possible since the glucose in the heart was a small fraction of the total observed.

\[13C\] and \[31P\] Spectra During Reflow

The changes observed in the \[13C\] and \[31P\] spectra for the same heart during the first 15 minutes of the 50-minute reflow period are shown in Figure 3. Labeled lactate, glycerol 3-phosphate, and succinate disappeared within 2–7 minutes after reflow; labeled alanine disappeared more slowly. Approximately 2–5 minutes after resumption of flow, glycogen synthesis resumed at a rate similar to the preischemic rate (Table 1). The changes observed in the 31P spectra (Figure 3B) during ischemia were largely reversed with resumption of flow. PCr rapidly returned to 95–100% of its preischemic intensity, internal Pi decreased, and acidosis was reversed. The Pi resonance became somewhat ill-defined during reflow, but after 2–5 minutes, its intensity and position were easily determined. The spectra obtained during reflow from GIK-treated hearts were similar to these shown for a control heart.

Correlation in Time of Carbon and Phosphorus Metabolites

The integrals from the \([1,13C]\)glycogen, \([3,13C]\)lactate, and \([3,13C]\)alanine resonances from the final 10 minutes of the preischemic period, during ischemia,
and during the first 20 minutes of the 50-minute reflow period are plotted versus time in Figure 4, from a representative experiment using 3 mM glucose and insulin. The figure also shows the corresponding changes in integrated intensities observed for PCr, Pi, β-ATP, and the intracellular pH observed in the 31P spectra. Notably, mobilization of glycogen continued for 4–5 minutes after the beginning of reflow. At this time, the PCr resonance had returned to 70–80% of its normoxic intensity. These observations on the time dependence and correlation of 13C and 31P spectra were qualitatively identical for both control and GIK-treated hearts. The presence of GDC in the perfusate did not significantly change the rates at which lactate, alanine, or Pi accumulated; the rates at which PCr and intracellular pH decreased during ischemia; or the rates at which these markers returned to their preischemic values during reflow. In the experiments in which GIK was added only at reflow (n = 3), lactate, alanine, Pi, PCr, and pH returned to their preischemic values during reflow at the same rates as in the control hearts and in the hearts treated with GIK from the beginning of the perfusion.

Glycogen, Lactate, and pH Changes: Comparison of GIK-Treated and Control Hearts

The rates of labeled glycogen synthesized during the preischemic period in the control and GIK-treated groups of hearts were similar, as shown in Table 1. The rates of labeled glycogen mobilized during ischemia are compared for control and GIK-treated hearts in Table 1. The rate for the GIK-treated hearts was about threefold lower than that observed in the control hearts. Also tabulated in Table 1 are the rates and amounts of lactate accumulation in the heart, detected by 13C NMR, and the amount of acidosis that occurred during the ischemic period. In contrast to the threefold difference between the control and GIK-treated hearts with respect to glycogen mobilization, the amounts and rates of labeled lactate formed and the pH changes observed during ischemia were similar in the two groups. Alanine formation was a constant fraction
Ala or Lac. Glyc. 

FIGURE 4. Changes in integrated intensities (arbitrary units) of selected $^{13}$C and $^{31}$P resonances and intracellular pH determined from the chemical shift of the Pi resonance during the final 10 minutes of the 40-minute normoxic period, 15 minutes of ischemia, and the first 20 minutes of the 50-minute reflow period in a heart perfused with insulin and 3 mM [1-$^{13}$C]glucose. Pi (○); pH (■); alanine (●); lactate (○). Ischemia was introduced at 10 minutes on the time axis (after 40 minutes of perfusion); reflow at 25 minutes (65 minutes after perfusion was begun).

Discussion

Synthesis of labeled glycogen from exogenous $^{13}$C-labeled glucose plus insulin proceeded at the same rate in the hearts perfused with 3 mM glucose as with GIK, suggesting that 3 mM glucose gave maximum glycogen synthesis rates in the presence of insulin under the conditions reported here. This finding is consistent with the action of insulin on glucose transport and the putative role of glycogen synthase as the rate-limiting step in glycogen synthesis.37

Our observations are consistent with the concept that all of the labeled glycogen synthesized during the labeling period is detectable by NMR despite the high molecular weight of this biopolymer. Experiments on isolated liver glycogen have shown that all24 or suggested that only a portion38 of the carbon in liver glycogen is detectable by high-resolution NMR. Our conclusions are not dependent on all of the labeled glycogen carbon being detectable by high-resolution NMR, only on the assumption that the same fraction is observed in both control and GIK-treated hearts. The ability to follow glycogen metabolism noninvasively, at 2-minute intervals, in a single perfused heart is a great advantage of $^{13}$C NMR. The alternative, to freeze-clamp numerous guinea pig hearts and assay them, is extremely laborious and requires a separate animal or biopsy for each time point. Moreover, in our laboratory and others, alkaline extraction procedures for assay of glycogen have given variable results.27 33

The $^{13}$C NMR spectra obtained during ischemia showed multiple products of anaerobic metabolism in cardiac muscle. In addition to lactate, the major product of anaerobic glycolysis, labeled alanine, succinate, and glycerol 3-phosphate accumulated. Accumulation of alanine and succinate is consistent with anaerobic metabolism of amino acids with the possible associated production of nucleoside triphosphates in the mitochondria.39 40 The accumulation of glycerol 3-phosphate is evidence for participation of the glycerol 3-phosphate dehydrogenase reaction in maintaining redox balance in the cytoplasm during ischemia.

The threefold lower mobilization rate of $^{13}$C-labeled glycogen observed during ischemia in the presence of GIK relative to that with 3 mM glucose is evidence for the proposed glycogen-sparing effect of GIK.4 Labeled lactate accumulation rate was similar in GIK-treated
and control hearts. We propose that glucose may supplement glycogen as the glycolytic substrate during ischemia in these experiments. The rates reported in Table 1 are initial rates. Since the relative contributions of glycogen and glucose to glycolysis are not constant throughout the 15-minute ischemic period, and since the glucose metabolized during ischemia is only a small fraction of the total observed by the NMR receiver coil, the exact amount of glucose needed for the glycogen-sparing effect cannot be precisely quantified from the present experiments. To demonstrate that this is a reasonable suggestion, we can estimate the amount of glucose available to these hearts during ischemia. Using estimates of extracellular space glucose41-43 plus intracellular glucose,42,43,44 the amount of glucose available (40-70 μmol/g dry wt) is in the range to account for the difference in glycogen mobilization in GIK-treated and control hearts. The use of glucose in place of glycogen during an ischemic event of limited duration is enigmatic since glycolysis from glucose yields 2 ATPs while that from glycogen yields 3 ATPs per glucose unit.

The similar rates and amounts of accumulation of labeled lactate and degree of acidosis observed for GIK-treated and control hearts suggests that GIK does not increase glycolysis under these conditions, in which the control contains 3 mM glucose and insulin in the perfusate. The similar pH change observed during ischemia for GIK-treated and control groups of hearts, in contrast with the threefold difference in labeled glycogen mobilized, is also consistent with supplementation of glycogen by glucose for glycolysis, resulting in similar amounts of ATP synthesized and hydrolyzed and similar acidosis in the two cases.45 These observations indicate that acidosis in muscle, in the presence of glucose, is not simply correlated with glycogenolysis. This result is different from that reported using glycogen-depleted hearts containing no glucose in the perfusate, in which the final ischemic pH was related to the glycogen content at the beginning of ischemia.29

The acquisition of simultaneous 13C and 31P NMR spectra allowed us to observe noninvasively that, upon reflow after ischemia, PCR synthesis resumed immediately while glycogenolysis continued for 4-5 minutes. Models for regulation of glycogen metabolism include regulatory roles for ATP, AMP, and P:33,35,46. If the levels of phosphorylated metabolites are highly correlated (i.e., a relatively constant total phosphate pool size), our observations support these concepts, in that glycogen mobilization does not stop nor glycogen synthesis resume until after the level of phosphorylated metabolites begins to return to preischemic levels. These observations also suggest that the role of these effectors on activities is kinetically rather than thermodynamically controlled. The degree to which different phosphorylated metabolites affect the flux of isotope is difficult to establish. During reflow, the Pi resonance became ill-defined so that determination of its integral during recovery after ischemia was not possible. Intracellular AMP concentrations are too low to detect by NMR spectroscopy. In addition, inferring specific regulatory mechanisms from correlations between levels of effectors and rates of pathways is not straightforward.47 An effector may control the flux of a pathway within a concentration range too small to be measured by NMR.

Our measurements of glycogen synthesis and recovery of PCR after ischemia were the same whether GIK was present from the beginning, given only at reflow, or absent. A working heart or in vivo preparation may show increased differences between controls and those treated with GIK. The unpaired Langendorff perfusion model we used gives a low cardiac workload. In response to ischemia, these hearts compensated for limited substrate and oxygen supply by reducing their rates of contraction, thus minimizing any ischemic effects. Some of the possible effects of GIK on metabolism may have been masked by the ability of the heart in this model to adjust its work output to match the substrate and oxygen supply. Extrapolation of the results reported here to a more physiologically relevant system or to clinical practice must be made with an understanding of the limitations of this model.

Results from the experiments reported here relate to two of the mechanisms postulated for GIK. The decreased mobilization rate of [1-13C]glycogen observed during ischemia in the presence of GIK indicates a glycogen-sparing effect. The rate of glycolysis, inferred from the appearance of labeled lactate and from extent of acidosis, was not significantly different in control and GIK-treated hearts, suggesting that GIK does not increase glycolysis during ischemia. Our observations suggest that even in total global ischemia, hearts may utilize exogenously supplied glucose in addition to glycogen as glycolytic substrate. The possible clinical benefits of GIK may be related to the ability of the ischemic heart to utilize exogenous glucose from the GIK mixture to supplement endogenous glycogen mobilization. Provision of GIK could delay glycogen depletion and thus prolong the production of high energy phosphate necessary for myocardial function during ischemia.

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