Protective Effect of Increased Glycolytic Substrate Against Systolic and Diastolic Dysfunction and Increased Coronary Resistance From Prolonged Global Underperfusion and Reperfusion in Isolated Rabbit Hearts Perfused With Erythrocyte Suspensions

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Current therapy of myocardial infarction may include early reperfusion. We simulated myocardial perfusion conditions during evolving myocardial infarction in isolated, normothermic, isovolumic rabbit hearts perfused with buffer containing bovine red blood cells (hematocrit of 40%), and we assessed the effects of high levels of glucose and insulin as “therapy” during prolonged (150-minute) severe underperfusion and reperfusion. Protocol 1 consisted of underperfusion at a constant coronary perfusion pressure of 8 mm Hg. The control group (n=8) received 5.5 mmol/l glucose and 15 microunits/ml insulin; the group treated with high levels of glucose and insulin (G+I) (n=8) received 19.5 mmol/l glucose and 250 microunits/ml insulin during both underperfusion and reperfusion. Relative to the control group, the G+I group experienced 1) greater developed pressure during underperfusion and increased recovery during reperfusion, 2) preserved diastolic function during underperfusion and reperfusion, 3) lower coronary resistance and greater coronary flow during the underperfusion period, 4) increased glycolytic flux and preserved glycogen stores and high energy phosphate levels, and 5) less loss of myocyte enzymes (creatine kinase and alanine aminotransferase). In protocol 2, coronary flow was kept identical in control (n=8) and G+I hearts (n=8) during the underperfusion period, and left ventricular end-diastolic pressure was kept below 10 mm Hg in both groups to minimize subendocardial damage and vascular compression. In this protocol, the effect of the G+I intervention in the prevention of an increase in coronary resistance during the underperfusion period was distinguished from its myocellular metabolic effects; the high G+I substrate had protective effects on mechanical and metabolic function that were less marked than, but similar to, those in protocol 1, indicating that its mechanisms of protection during underperfusion affected both cardiac function and coronary resistance. We conclude that the G+I intervention, in clinically relevant concentrations, markedly protected severely underperfused myocardium for 150 minutes and may be a beneficial intervention in combination with reperfusion therapy in acute myocardial infarction. (Circulation Research 1991;68:466–481)

Reperfusion therapy of acute myocardial infarction is limited by the ischemic damage that occurs before reperfusion can be instituted, by the increased resistance to coronary flow (the “no-reflow” phenomenon) that occurs after prolonged ischemic injury, and by possible “reperfusion injury.” The recipient of a grant for young investigators of the Swiss National Foundation of Science.

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sion injury” when myocytes that are viable before underperfusion are irreversibly injured by the reperfusion process itself.\textsuperscript{1,2} Intervention with high levels of glucose and insulin (G+I) has the potential to protect against ischemic myocardial injury by enhancing glycolytic flux; however, despite 35 years of investigation, the role of glycolysis remains controversial. Under conditions of no-flow or severe underperfusion, when stimulation of glycolysis results in increased tissue lactate accumulation, it has been associated with increased tissue injury.\textsuperscript{3} In contrast, if lactate accumulation is prevented by a low level of ischemic perfusion that permits lactate diffusion, results from numerous studies are consistent with a protective effect of an augmented glycolytic flux on hypoxic or partially ischemic myocardial tissue.\textsuperscript{4–9}

Under conditions of acute myocardial infarction, a certain degree of residual and collateral flow occurs\textsuperscript{10,11} and may be further enhanced by thrombolytic therapy, providing the rationale for a metabolic intervention.

Evidence supporting a role for glycolysis in maintaining cell viability during underperfusion has recently been reviewed.\textsuperscript{12} Recent studies have shown that glycolytically derived ATP is of particular importance in the preservation of cell membrane function and cell integrity,\textsuperscript{13–16} whereas ATP from oxidative phosphorylation preferentially supports contractile function.\textsuperscript{17} Exogenous glucose has been identified as a superior substrate, relative to glycogen and oxidative substrates, in the prevention of ischemic cardiac injury.\textsuperscript{18,19} Glycolysis appears to be of special importance in protecting hearts with left ventricular hypertrophy against hypoxic injury.\textsuperscript{20} In addition, preliminary studies\textsuperscript{21,22} have suggested a protective effect of enhanced glycolysis during reperfusion. However, no previous study has tested the effect of glycolysis on coronary resistance during underperfusion and the no-reflow phenomenon during reperfusion.

We were motivated to study G+I intervention after a review of the literature revealed that no investigation had thoroughly assessed its functional and metabolic effects in a protocol that simulated the perfusion conditions and metabolic milieu of an acute myocardial infarction treated with early reperfusion. We established a model in which the entire left ventricle in an isolated rabbit heart, perfused with a suspension of erythrocytes at a hematocrit of 40%, is subjected for 150 minutes to the degree of underperfusion present in an acute infarct region. We studied the effects of G+I intervention on both myocardial and coronary vascular function during underperfusion and reperfusion (30 minutes). To simulate the myocardial metabolic milieu present in patients with acute infarction, the coronary perfusate for the control group of rabbits had clinically normal values for hematocrit and oxygen content and normal levels of glucose, insulin, and free fatty acid. In the G+I group, glucose and insulin levels were increased to values achieved during clinical G+I trials. Our results suggest that G+I intervention has the potential to increase the benefits of reperfusion therapy of acute myocardial infarction.

Materials and Methods

Experimental Preparation

We used an isolated, isovolumically beating rabbit heart preparation that was perfused with modified Krebs-Henseleit buffer containing bovine red blood cells at a hematocrit of 40%. This model has been described in detail previously\textsuperscript{23,24} and is illustrated in Figure 1. After administration of 75 mg/kg pentobarbital sodium and heparin (1,000 units), hearts of male New Zealand White rabbits (1.5–2.0 kg) were excised and perfused within 10 seconds of excision. A cannulated, collapsed thin-walled latex balloon was placed in the left ventricle via the left atrium and secured by means of several suture ties around the left atrial tissue cuff, which contained the cannula from the left ventricular balloon. The balloon was connected to a Statham P23dB pressure transducer (Gould, Oxnard, Calif.) to measure left ventricular pressure and its first derivative. The fidelity of our pressure recording system satisfies the criteria required for accurate measurement\textsuperscript{25} and has been previously reported.\textsuperscript{26} The heart was placed in a
water-jacketed constant-temperature chamber and submerged in saline as shown in Figure 1.

A red blood cell perfusate similar to that described by Marshall and Zhang\(^\text{27}\) was used, consisting of bovine red blood cells at a final hematocrit of 40% in Krebs-Henseleit buffer. Fresh whole cow blood was collected at a local slaughterhouse into a vessel containing 15,000 units sodium heparin/l, immediately placed on ice, and then centrifuged at 5°C at 3,000 rpm for 15 minutes. The supernate was aspirated, and the resulting packed cells were mixed 1:1 with Krebs-Henseleit buffer. The centrifugation and resuspension steps were repeated three times. The red blood cell suspension was thus essentially white blood cell and platelet free. For future use, packed red blood cells were stored at 4°C and washed daily.

The Krebs-Henseleit buffer contained (mmol/l) NaCl, KCl, CaCl\(_2\), KH\(_2\)PO\(_4\), MgSO\(_4\), NaHCO\(_3\), glucose, lactate, palmitic acid (as a source for fatty acid) 0.4, together with 4% bovine serum albumin (Sigma Chemical Co., St. Louis). Essentially free fatty acid–free bovine serum albumin (No. A-7030, Sigma) was first dissolved in Krebs-Henseleit buffer. Palmitic acid (No. P-9767, Sigma) was then added to this mixture. The buffer was prepared fresh daily. Gentamicin (0.2 mg/dl) was added to the red blood cell perfusate to retard bacterial growth. The perfusate was equilibrated with 20% O\(_2\)-3% CO\(_2\)-77% N\(_2\) to achieve a PO\(_2\) of 140 mm Hg and a pH of 7.40.

Data Collection and Analytical Procedures

Pressure measurements were recorded continuously. In experiments in which left ventricular balloon volume was kept constant (see protocol 1, below), an increase in left ventricular end-diastolic pressure (LVEDP) signified a decrease in diastolic chamber distensibility.\(^\text{26,28}\) In experiments in which LVEDP was held constant (by decreasing left ventricular balloon volume when contracture occurred, as in protocol 2, below), left ventricular diastolic chamber distensibility was assessed by generating a diastolic pressure–volume filling curve at the end of the baseline period and at the end of the reperfusion period. Left ventricular diastolic pressure–volume filling curves were obtained by increasing left ventricular balloon volume by 0.2-ml increments up to a volume that produced an end-diastolic pressure of 30–35 mm Hg. For comparison of diastolic stiffness between groups, left ventricular volumes were determined from each pressure–volume curve at 5 mm Hg pressure intervals as previously described.\(^\text{29}\)

Coronary blood flow was measured by timed collections of the venous effluent. Myocardial oxygen consumption was calculated from the arteriovenous oxygen content differences, derived from oxygen saturation curves for the Krebs buffer–red blood cell suspensions over the experimental range of pH and PO\(_2\) values. Venous and arterial creatine kinase (creatinine-N-phosphotransferase, EC 2.7.3.2) and alanine aminotransferase (l-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2) activities and glucose concentration were measured using the COBAS BIO system (Roche Diagnostic Systems, Nutley, N.J.). For lactate assays, samples were diluted 1:2 with 10% trichloroacetic acid and stored at 4°C until analyzed by a specific enzymatic method.\(^\text{30}\)

At the end of the experiment, hearts were freeze-clamped with aluminum tongs precooled in liquid nitrogen, and tissue levels of ATP, creatine phosphate, and lactate were determined spectrophotometrically. Glycogen levels were measured using an enzymatic technique.\(^\text{33}\) Measurements are expressed as micromoles per gram left ventricular dry weight.

### Stability of the Isolated Blood-Perfused Rabbit Heart Preparation Under Well-Oxygenated Conditions

To determine the mechanical and metabolic stability of the isolated heart during prolonged perfusion with suspended red blood cells, six hearts were perfused at a coronary perfusion pressure of 100 mm Hg for 3.5 hours. After the initial 30-minute equilibration period, developed pressure declined by only 14% during the subsequent 3 hours (Table 1). With the left ventricular balloon volume held constant, there was a slight decrease in LVEDP and maximal negative dP/dt. Coronary blood flow decreased to 73±0.07% of initial flow, indicating a small increase in coronary resistance. The hearts extracted approximately 16% of the arterial lactate level. Mean lactate uptake was 96±52 \(\mu\)mol/g dry wt/180 min. There was a minimal release of creatine kinase (average overall production = 78±26 units/g dry wt/180 min) and alanine aminotransferase (average overall production = 582±162 milliunits/g dry wt/180 min). By the periodic addition of glucose to the venous reservoir, the arterial glucose level was maintained constant at 4.99±0.52 mmol/l. Glucose utilization was 465±153 \(\mu\)mol/g dry wt/180 min during the 3-hour perfusion period. The myocardial content

### Table 1. Hemodynamic Data of Six Oxygenated Control Hearts at Baseline and After 180 Minutes of Perfusion With Suspended Red Blood Cells

<table>
<thead>
<tr>
<th></th>
<th>LVDP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>Max dP/dt (mm Hg/sec)</th>
<th>Min dP/dt (mm Hg/sec)</th>
<th>CBF (ml/min/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>92.0±7.2</td>
<td>9.2±0.9</td>
<td>1,528±211</td>
<td>1,081±92</td>
<td>6.2±1.71</td>
</tr>
<tr>
<td>After perfusion (180-min)</td>
<td>79.5±8.2*</td>
<td>7.2±0.7*</td>
<td>1,456±172</td>
<td>860±59*</td>
<td>4.2±0.8*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; Max dP/dt, left ventricular maximal positive dP/dt; Min dP/dt, left ventricular maximal negative dP/dt; CBF, coronary blood flow.

\(^*p<0.01\) compared with baseline measurements.
of ATP, creatine phosphate, and glycogen was stable (Table 2). These results demonstrate that the preparation is functionally and metabolically stable for the length of time required for the experimental protocols described below.

**Experimental Protocols**

In two protocols of severe underperfusion for 150 minutes followed by 30 minutes of reperfusion, the control group was compared with the G+I treatment group, which was perfused with high glucose and insulin during both underperfusion and reperfusion. In control hearts, the initial concentrations of 5.5 mmol/l glucose and 15 microunits/ml insulin (regular beef/pork insulin, Iletin, Eli Lilly Co., Indianapolis, Ind.) were maintained throughout the protocol by the addition of glucose to the perfusate. Comparable insulin levels have been reported during myocardial infarction in humans (range, 15–25 microunits/ml).34–36 In the G+I group, at 5 minutes after the onset of underperfusion, glucose and insulin were added to increase their concentrations to 19.5 mmol/l and 250 microunits/ml, respectively. These concentrations were selected because they have been observed during G+I infusions in clinical trials.37–41

During a 30-minute baseline equilibration period, all hearts were perfused at a coronary perfusion pressure of 100 mm Hg. Left ventricular balloon volume was adjusted to achieve an LVEDP of 8–10 mm Hg. Hearts were paced at a rate of 180 beats/min throughout the whole experiment. During the equilibration period, the hearts achieved a hemodynamic steady state, and baseline hemodynamic and metabolic measurements were performed.

**Protocol 1.** In a first protocol, the coronary perfusion pressure was reduced to 8 mm Hg for 150 minutes to impose severe underperfusion in both control and G+I groups. Left ventricular balloon volume was held constant throughout the experiment such that changes in LVEDP reflected changes in left ventricular diastolic distensibility.26 After 150 minutes of underperfusion the coronary perfusion pressure was readjusted to the baseline value of 100 mm Hg, and the hearts were reperfused for 30 minutes. A 30-minute reperfusion period was chosen after preliminary experiments showed that recovery of mechanical function stabilized within this period. Arterial and venous samples were collected throughout the underperfusion and reperfusion periods.

**Protocol 2.** Because the results from protocol 1 demonstrated a progressive increase in LVEDP and a decrease in coronary flow in the control group during the underperfusion period, we performed a second protocol in which these two parameters were held constant. In the second protocol, the control and the G+I groups received the same constant flow rate during the underperfusion period, in contrast to the constant perfusion pressure provided in protocol 1. In addition, in protocol 2 a constant LVEDP of 10 mm Hg was maintained throughout the underperfusion/reperfusion period in both groups to prevent any subendocardial compression or damage. Coronary flow was monitored throughout the underperfusion period, and perfusion pressure was increased in the control group as needed to maintain coronary flow equal to that of the G+I group. LVEDP was held constant at 10 mm Hg throughout the underperfusion period by gradually reducing left ventricular balloon volume when contracture developed. During the first 10 minutes of reperfusion, the left ventricular balloon was emptied in both groups and then continuously adjusted to maintain an LVEDP of 10 mm Hg for the remaining 20 minutes of reperfusion. Thus, any differences in the extent of injury between the control and G+I groups in protocol 2 cannot be attributed to differences in ischemic coronary flow or LVEDP per se during underperfusion, since these parameters were held constant and equal in both groups. Each experimental group in each protocol consisted of eight hearts.

**Statistical Analysis**

Data are reported as mean±SEM. Data acquired sequentially in individual hearts were tested by anal-
ysis of variance for repeated measures. Comparison between two experimental groups was performed by two-way analysis of variance. If overall analysis of variance indicated a significant difference of groups, trials, or interaction, values at specific time points were examined by the method of least significant differences. A difference of a single metabolic measurement between experimental groups within one protocol was tested by the unpaired t test. When a comparison among oxygenated controls and both groups of an experimental protocol was made, one-way analysis of variance was used. A value of \( p < 0.05 \) was considered significant.

**Results**

**Protocol 1: Constant Perfusion Pressure (8 mm Hg) and Constant Left Ventricular Balloon Volume During Underperfusion**

**Systolic function.** Baseline left ventricular developed pressure in the control and G+I groups was virtually the same (95 ± 4 versus 94 ± 7 mm Hg) (Figure 2). After 15 minutes of underperfusion, the G+I group maintained a significantly better developed pressure than the control group. By the end of the underperfusion period, the developed pressure of the control hearts had decreased to 5 ± 1 mm Hg (5.2 ± 1.4% of baseline), but the G+I hearts had a developed pressure of 23 ± 2 mm Hg (24.7 ± 2.4% of baseline, \( p < 0.01 \) versus controls). After 30 minutes of reperfusion, the control group recovered to only 17.3 ± 5.9%, but the G+I group recovered to 68.4 ± 2.0% of initial developed pressure (\( p < 0.01 \)). The decrease and recovery of maximal positive and negative dP/dt during underperfusion and reperfusion showed differences between the control and G+I groups similar to the differences in developed pressure. Thus, the G+I intervention markedly protected systolic function during underperfusion and after recovery.

**Diastolic function.** Since left ventricular balloon volume was held constant in protocol 1, an increase in LVEDP reflected an increase in diastolic chamber stiffness or “contracture.” At the end of the underperfusion period, LVEDP increased by 31 ± 3 mm Hg in control hearts, whereas the increase in the G+I group was only 2 ± 1 mm Hg (\( p < 0.01 \)) (Figures 2 and 3). Within the first 5 minutes of reperfusion, LVEDP increased to 90 ± 5 mm Hg in the control group but only to 39 ± 5 mm Hg in the G+I group (\( p < 0.01 \)). At the end of reperfusion LVEDP was 62 ± 7 versus 23 ± 5 mm Hg (\( p < 0.01 \)) in the control and G+I groups, respectively (Figure 2). Thus, significantly less chamber stiffness increase occurred in the G+I group than in the control group during both underperfusion and reperfusion.

**Enzyme leakage.** The G+I group had less cellular damage as assessed by creatine kinase and alanine aminotransferase release (Figure 4). Cumulative creatine kinase release during the entire underperfusion/reperfusion protocol was 902 ± 191 units/g dry wt in control hearts versus 314 ± 65 units/g dry wt in hearts of the G+I group (\( p < 0.05 \)). Overall alanine aminotransferase release was 4,176 ± 788 versus 2,066 ± 839 milliunits/g dry wt (\( p < 0.05 \)) in the control versus G+I groups. These values were severalfold higher than the rate of enzyme leakage observed in a
comparable period of well-oxygenated perfusion (see "Materials and Methods"), indicating significant myocardial injury in this underperfusion/reperfusion protocol and significant protection with the G+I substrate relative to the controls.

Coronary resistance changes. Baseline coronary flow was similar in both groups before the underperfusion period and was in the physiological range for a normal in vivo rabbit heart (5.37±0.56 versus 5.53±0.89 ml/min/g dry wt for control versus G+I groups). At the onset of underperfusion, coronary flow decreased to 11–13% of baseline in both groups. During the underperfusion period in the control group, a steady decline in coronary flow from 11.3±2.0% to 2.2±0.5% (p<0.01) of initial baseline flow was observed (Figure 5). However, in the G+I group during the underperfusion period, coronary flow decreased only slightly, from 13.1±1.7% to 11.0±1.4% (p<0.05) of initial blood flow. The difference in coronary flow during the underperfusion period between the control and G+I groups was significant (p<0.01). Hyperemia after underperfusion was significantly greater in the G+I group than in the control group (Figure 5). Thus, the G+I treatment was associated with better myocardial perfusion relative to the control group during underperfusion and early reperfusion, despite the identical coronary perfusion pressure in both groups.

The progressive increase in coronary resistance and decrease in coronary flow during underperfusion in the control group occurred in parallel with the increase in LVEDP (see Figures 3 and 5). Conversely, in the G+I group, in which no significant increase in LVEDP occurred during underperfusion, there was a minimal decrease in coronary flow during the underperfusion period.

The enhanced coronary flow in the G+I group during underperfusion was "nutritional" and not simply arteriovenous shunting, since it resulted in greater oxygen consumption during underperfusion in the G+I group relative to the control group (Figure 6). Overall oxygen consumption during the entire underperfusion period was 203±49 versus 531±77 μmol/g dry wt in the control versus G+I groups (p<0.01).

The relation between oxygen consumption and mechanical function reflects overall metabolic/mechanical efficiency and is a measure of ischemic injury. In these experiments, because contraction was isovolumic and heart rate was held constant, the ratio of developed pressure to oxygen consumption can serve as an index of myocardial efficiency. During
underperfusion, the ratio of oxygen consumption to developed pressure was not different between the two groups, but during reperfusion, control hearts used significantly more oxygen relative to developed pressure. At 15 minutes of reperfusion, the ratio of oxygen consumption/developed pressure was 0.473±0.083 versus 0.215±0.022 μmol/min/g dry wt/mm Hg for the control versus G+I groups (p<0.01), and at 30 minutes of reperfusion, it was 0.409±0.069 versus 0.214±0.024 μmol/min/g dry wt/mm Hg (p<0.025). Thus, the G+I group had better overall metabolic/mechanical efficiency than the control group during reperfusion.

Glycolytic flux. Glycolytic flux was assessed by measuring glucose uptake, glycogen depletion, and lactate production. During the baseline period, both groups demonstrated net lactate uptake, indicating an aerobic pattern of metabolism. With the onset of underperfusion, net lactate production promptly occurred (Figures 3 and 7). After 30 minutes of underperfusion in the control group, lactate production began to decrease, and after 75 minutes, it virtually stopped. However, the G+I group maintained the initial relatively high level of lactate production throughout the 150 minutes of underperfusion.

During underperfusion and reperfusion, total lactate production, calculated by integrating the area under the washout curve, was 119±24 μmol/g dry wt in the control group and 270±50 μmol/g dry wt in the G+I group (p<0.01) (Figure 7). However, tissue lactate accumulation measured at the end of reperfusion did not differ between the two groups (17.8±1.9 versus 16.5±3.2 μmol/g dry wt) (Table 2). The rates of lactate production during underperfusion appeared to be related to changes in diastolic chamber stiffness and contracture. Concomitant with the decrease in lactate production after 30 minutes of underperfusion in the control group, LVEDP started to increase and reached its plateau after 75 minutes, when lactate production virtually stopped (Figure 3). In the G+I group, the initial high rate of lactate production was maintained throughout the whole underperfusion period and was associated with only a minimal increase of LVEDP (2±1 mm Hg).
The G+I group had greater glucose uptake than did the control group during both underperfusion and reperfusion (Figure 7). Cumulative glucose uptake during underperfusion was 91 ± 16 μmol/g dry wt in the control group compared with 386 ± 59 μmol/g dry wt in the G+I group (p < 0.01). This greater uptake of exogenous glycolytic substrate in the G+I group relative to the control group was reflected in the utilization of the endogenous glycolytic substrate, glycogen; control hearts depleted 90% of their initial glycogen content, whereas the G+I group preserved 50% of their glycogen content (p < 0.01) (Table 2). Overall, the cumulative utilization of glycolytic substrate (glucose uptake plus glycogen depletion) was twofold greater in the G+I group than in the control group, as was the cumulative lactate production.

The greater glycolytic flux and oxygen consumption values of the G+I group during underperfusion and reperfusion were associated with a better preservation of high-energy phosphates (ATP and creatine phosphate) relative to the control group at the end of reperfusion (Table 2).

Protocol 2: Constant Coronary Flow and LVEDP During Underperfusion

In protocol 1, despite identical coronary perfusion pressures during underperfusion in both groups, the G+I intervention decreased the severity of underperfusion, since coronary flow was greater in the G+I group than in the control group. We hypothesized that relative subendocardial perfusion was probably also different in the two groups, since the progressive rise in LVEDP in the control group probably compromised subendocardial flow, but such subendocardial vascular compression would not have occurred in the G+I group, in which LVEDP did not increase during the underperfusion period.

To separate any perfusion effects of the G+I intervention from its myocyte metabolic effects, in protocol 2 we compared the G+I and control groups during a period of identical severity of underperfusion. Coronary perfusion pressure was increased as necessary in the control group to compensate for an increase in coronary resistance and to maintain comparable coronary flows during the underperfusion period in both groups. To minimize any difference in subendocardial compression between the two groups, LVEDP was closely monitored, and volume was withdrawn as necessary from the intra–left ventricular balloon in the control group to compensate for contracture and to maintain a constant LVEDP of 10 mm Hg in both groups throughout the underperfusion period.

To provide comparable coronary flow levels during underperfusion in the control and G+I groups (Figure 5, protocol 2), it was necessary to progressively
increase coronary perfusion pressure in the control group from 8.0±0.1 mm Hg at the onset of underperfusion to 15.1±2.1 mm Hg at the end of the underperfusion period (p<0.01). In contrast, coronary perfusion pressure remained at 8 mm Hg during the entire underperfusion period in the G+I group. Baseline coronary flow was similar in the two groups (5.27±0.45 versus 5.42±0.5 ml/min/g dry wt). Coronary flow decreased to 17.2±2.5% versus 19.9±1.1% (p=NS) of baseline in the control and G+I groups, respectively, after 15 minutes of underperfusion and to 11.0±0.8% versus 12.9±2.0% (control group versus G+I group; p=NS) at the end of the underperfusion period. The regulation of coronary flow during underperfusion in protocol 2 was successful in providing the same degree of oxygenation to both groups as judged by myocardial oxygen consumption (Figure 6). During the underperfusion period, cumulative oxygen consumption was 504±59 μmol/g dry wt in the controls and 643±55 μmol/g dry wt in the G+I group (p=NS).

To maintain a constant LVEDP of 10 mm Hg during underperfusion in the controls, it was necessary to decrease the LV balloon volume during the underperfusion period from 1.34±0.06 ml at the onset of underperfusion to 0.78±0.09 ml (p<0.01) at the end of underperfusion (Figure 3, protocol 2). However, no alteration of balloon volume was required in the G+I group since LVEDP did not increase in this group during underperfusion.

**Systolic function.** Despite the similar coronary flows and constant LVEDP of 10 mm Hg in both groups, left ventricular developed pressure was still significantly higher during underperfusion in the G+I group than in the control group (Figure 8). Control hearts recovered to only 54±4% of baseline developed pressure, whereas hearts treated with glucose and insulin recovered to 83±5% (p<0.01). A similar greater recovery of maximal positive and negative dP/dt was observed in the G+I group relative to controls, but only the maximal negative dP/dt intergroup difference achieved statistical significance. There was no difference in metabolic/mechanical efficiency (oxygen consumption/developed pressure) between the two groups during underperfusion or reperfusion.

**Diastolic function.** The progressive decrease in LV balloon volume required in the control group to maintain LVEDP at 10 mm Hg during underperfusion is a measure of the increase in diastolic chamber stiffness or contracture which occurred (Figure 3). Diastolic filling curves are shown in Figure 9. At baseline, before underperfusion, the pressure–volume diastolic filling curves were virtually identical in the two groups. However, at the end of reperfusion the control group’s curve had shifted more markedly to the left (p<0.01) and had a steeper slope than that of the G+I group (p<0.05), indicating a greater increase in diastolic chamber stiffness in the control group than in the G+I group.

**Glycolytic metabolism.** As in protocol 1, the onset of contracture (decrease in left ventricular balloon volume) in the control group in protocol 2 occurred concomitantly with the decrease of lactate production after 30 minutes of underperfusion (Figure 3). The G+I group maintained a steady level of lactate production and developed no contracture during underperfusion. Cumulative lactate washout during underperfusion and reperfusion (Figure 10) was lower in the control group than in the G+I group (129±19 versus 270±9 μmol/g dry wt; p<0.01), but tissue lactate accumulation at the end of reperfusion did not differ (18.6±1.9 versus 14.7±2.5 μmol/g dry wt; p=NS). Consonant with the higher lactate pro-
The position.

During underperfusion and at the end of reperfusion (protocol 2). Diastolic pressure-volume filling curves were obtained in each heart before underperfusion (control: group treated with high levels of glucose and insulin; n=8 hearts in each group) and at end of reperfusion (control: group treated with high levels of glucose and insulin (G+I)). Left ventricular (LV) balloon volume was increased in small increments to progressively increase LV end-diastolic pressure to 30 mm Hg. To compare the pressure-volume relation for two groups of hearts at common LV end-diastolic pressure values, the LV volumes at every 5 mm Hg of LV end-diastolic pressure from 5 to 30 mm Hg were read from each pressure-volume curve to derive the mean LV volume (±SEM) at each LV end-diastolic pressure. After underperfusion and reperfusion, the pressure-volume curves of the control and G+I groups both shifted significantly to the left relative to their preischemic position. The control hearts shifted further than the G+I hearts (p<0.001), indicating a greater decrease in diastolic distensibility in the control group than in the G+I group.

Production was a significantly higher glucose uptake in the G+I group compared with the control group during underperfusion and early reperfusion (Figure 10). Total glucose uptake during underperfusion was 175±17 in the control group compared with 603±40 μmol/g dry wt in the G+I group (p<0.01). As in protocol 1, the lesser utilization of exogenous glucose in the control group resulted in less preservation of endogenous glycogen (43%) compared with the G+I group (83%) (p<0.01) (see Table 2). In contrast with protocol 1, the tissue contents of ATP and creatine phosphate were equal in the two groups of protocol 2 at the end of the reperfusion period (Table 2).

Enzyme release. Cellular damage in protocol 2 was also assessed by the amount of enzyme release. Although creatine kinase and alanine aminotransferase washout occurred later during reperfusion in the control group compared with the G+I group (p<0.01), the cumulative amount of enzyme release did not differ between the G+I and control groups for creatine kinase (505±95 versus 334±87 units/g dry wt; p=NS) or for alanine aminotransferase (4,540±839 versus 3,460±870 milliunits/g dry wt; p=NS) (Figure 11).

Subgroups in protocol 2: Groups with identical oxygen consumption during underperfusion. The goal of protocol 2 was to impose an equal and constant degree of underperfusion in the control and G+I groups. This goal was achieved in terms of coronary flow (Figure 5). However, even though coronary flow and oxygen consumption values (Figure 6) were not statistically different between the two groups, a non-significant trend was present, indicating slightly greater oxygen consumption and oxidative metabolism in the G+I group relative to the control group (643±35 versus 504±59 μmol O2/g dry wt [p=NS] for cumulative oxygen consumption during the 180-minute underperfusion period). This intergroup difference of 0.77 μmol O2/g dry wt/min was relatively small, equivalent to approximately 4% of baseline oxygen consumption. Nonetheless, this small amount of greater oxidative metabolism, rather than the increased glycolytic flux, could possibly have been responsible for the G+I group’s better mechanical function during and after underperfusion. Therefore, to definitively separate the effects of glycolytic and oxidative metabolism in protocol 2, we retrospectively defined control and G+I subgroups with precisely matched oxygen consumption values during the underperfusion period (565±59 versus 575±40 μmol O2/g dry wt/180 min; n=6 per group).

Comparison of these subgroups with matched oxygen consumption reaffirmed the superiority of G+I substrate relative to the control group. During the
In these experiments, the isolated rabbit heart, perfused with an erythrocyte suspension, was subjected to severe sustained underperfusion for 150 minutes in a protocol designed to simulate the perfusion conditions and metabolic milieu in the region of an acute myocardial infarction. During the period of underperfusion, the myocardial flow rate of approximately 0.1 ml/min/g wet wt was comparable with values measured in experimental studies of acute canine myocardial infarction.\textsuperscript{10,11} The control perfusate contained both major myocardial metabolic substrates (free fatty acid and glucose) and insulin in concentrations found in patients with acute myocardial infarction; the high glucose and insulin concentrations were comparable with levels observed in the blood of patients treated with clinical high glucose and insulin protocols.\textsuperscript{37–41}

Under our experimental conditions, G+I treatment markedly protected the underperfused myocardium. A major component of the mechanism of protection was the maintenance of a low coronary resistance in the G+I group. Thus, in protocol 1, which maintained a constant coronary perfusion pressure of 8 mm Hg, myocardial perfusion did not progressively deteriorate in the G+I group as it did in the control group, and the higher perfusion level in the G+I group was associated with a significant reduction in the severity of injury. In protocol 1, relative to the control group, the G+I group exhibited better contractile function during underperfusion and after reperfusion, no ischemic increase in diastolic chamber stiffness, less loss of cellular enzymes, and greater preservation of myocardial high-energy phosphate and glycogen levels at the end of the protocol. These results are consistent with previous observations indicating that very small increases in myocardial perfusion levels during underperfusion can have a marked effect in reducing ischemic myocardial injury.\textsuperscript{43}

To determine whether all of the beneficial effects observed with G+I intervention in protocol 1 could be attributed to the increased myocardial perfusion and resultant increase in oxidative metabolism and tissue washout during underperfusion, we performed protocol 2, in which myocardial perfusion rates, oxygen consumption, and LVEDP were maintained at comparable values during underperfusion in both groups. In contrast to protocol 1, there were no significant intergroup differences in the preservation of tissue high-energy phosphate levels or in the loss of cellular enzymes during the underperfusion and reperfusion periods of protocol 2. Thus, the protective effect of the G+I treatment on these parameters appears to be dependent on the increase in myocardial perfusion and oxidative metabolism. However, in protocol 2, the G+I group exhibited significantly better contractile function during underperfusion and after reperfusion, less increase in diastolic chamber stiffness, and greater preservation of myocardial
glycogen stores than did the control group. Thus, these parameters appear to benefit directly from the increased glycolytic flux associated with the high G+I substrate. A subgroup analysis of six hearts from each group in protocol 2, matched for identical subgroup oxygen consumption values, confirmed the protective effect of increased glycolytic flux on both systolic and diastolic function with underperfusion and reperfusion, independent of any influence of oxidative metabolism. The combined results of protocols 1 and 2 indicate that the G+I intervention protects the ischemic myocardium by two separate mechanisms—improved myocardial perfusion and preservation of myocyte function and integrity.

Glycolytic flux and ischemic contracture were closely linked. In both protocol 1 and protocol 2, the increase in diastolic chamber stiffness (contracture) during underperfusion occurred in parallel with a decrease in glycolytic flux, as assessed by lactate production (Figure 3). Similarly, the progressive reduction in coronary flow during underperfusion in the protocol 1 control group occurred concomitantly with the increase in LVEDP in that group and may have been partly related to the LVEDP increase per se. However, the increased LVEDP was not completely responsible for the decrease in myocardial perfusion during underperfusion in the protocol 1 control group, because in protocol 2 LVEDP was not allowed to increase above 10 mm Hg, but it was still necessary to increase the coronary perfusion pressure in the control group during the underperfusion period to maintain equal coronary flow in the control and G+I groups. Thus, there appeared to be a mechanism that increased coronary resistance in the control group independent of an increase in LVEDP, which was prevented by the enhanced glycolytic flux.

Limitations of the Model

Our experimental protocols and model were designed to simulate the myocardial perfusion conditions and the metabolic milieu in an acutely infarcting myocardial region, but our experimental design had some noteworthy differences from an acute infarction. We imposed global underperfusion to be able to control precisely the degree of perfusion, but an acute infarct results from regional ischemia. We used a perfusate of a red blood cell suspension lacking the leukocytes and platelets that can contribute to the pathophysiology of ischemic processes. In protocol 2, we maintained LVEDP equal to 10 mm Hg in both the control and G+I groups and assumed that similar LVEDP values would result in a similar degree of subendocardial compression in both groups. Stein et al.48 have shown that diastolic intramyocardial pressures are higher than diastolic intracavitary ventricular pressures and that an epicardial to endocardial transmural diastolic pressure gradient of approximately 15 mm Hg is present in the normal canine heart. Thus, in protocol 2, intramyocardial pressures probably exceeded the left ventricular intracavitary diastolic pressure of 10 mm Hg; myocardial contracture and edema may have contributed to intramyocardial pressure during underperfusion, and it is likely that the flow distribution was not homogeneous throughout the myocardium despite a constant LVEDP of 10 mm Hg. Nonetheless, in protocol 2, the control and G+I groups had comparable values of coronary flow and oxygen consumption, and subgroups had virtually identical oxygen consumption values yet differed markedly in mechanical function, depending on the presence of the G+I substrate. The equivalent amounts of oxygen consumption in the control and G+I groups during underperfusion in protocol 2 support the conclusion that myocardial perfusion was comparable in these groups and thus support the conclusion that the beneficial effects of the G+I substrate in protocol 2 resulted from protective actions on the myocyte independent of an effect on coronary resistance.

Mechanism of Action of High Glucose and Insulin Substrate

There are several mechanisms by which the G+I intervention could have improved ischemic and postischemic myocardial and vascular function; these have been nicely summarized by Opie45 and include replenishment of potassium ion, reduction of ischemic injury by increasing glycolytic flux and glycolytic ATP synthesis, esterification of intracellular free fatty acids by increasing the supply of α-glycerophosphate, and increasing the blood osmolarity level. Hess et al.46 have also proposed that G+I intervention may be protective by a mechanism of scavenging free radicals, which would thereby protect sarcoplasmic reticular calcium uptake function. It is likely that more than one mechanism may be responsible for the beneficial effects of the G+I intervention that we have observed on the coronary vasculature and on the myocardium.

Role of Glycolytic Flux

Although not excluding other mechanisms of action, our results are consistent with numerous studies9,19,45,47-49 demonstrating an association between the rate of glycolytic flux and anti-ischemic or anti-hypoxic protection. Owen et al.19 have recently studied the relation between ischemic contracture and the source and rate of ATP production, comparing glucose flux, glycogenolysis, and oxidative phosphorylation in isolated buffer-perfused rat hearts subjected to global underperfusion. The rate of glycolytic flux from glucose was the metabolic parameter that correlated best with prevention or delay of ischemic contracture during 45 minutes of global low-flow ischemia.

Consonant with Owen et al.,19 we observed no increase in diastolic chamber stiffness (i.e., no contracture) during underperfusion in the hearts that received the high G+I substrate. In protocol 2, the control and G+I groups had comparable rates of oxygen consumption that were 20–25% of baseline (Figure 6) and that would be expected to generate
approximately 24 μmol ATP/g dry wt/min or 3,600 μmol ATP/g dry wt during 150 minutes of underperfusion (assuming a phosphorus/oxygen ratio of 3 and assuming that respiration remained coupled during low flow ischemia), yet ischemic contracture occurred unless the G+I substrate was present. Quantitatively, the increased glycolytic flux associated with the G+I substrate would not be expected to greatly increase total ATP synthesis above the level supported by continued oxidative phosphorylation at 20–25% of baseline. For example, in protocol 2, assuming 2 ATP/mol glucose uptake and 3 ATP/mol glycogen utilization during underperfusion, glycolytic ATP production would amount to approximately 512 μmol ATP/g dry wt/150 min of underperfusion in the control group and 1,260 μmol ATP/g dry wt/150 min of underperfusion in the G+I group. Thus, the G+I substrate increased total ATP production from approximately 4,112 to 4,860 μmol/g dry wt/150 min or by approximately 18%. However, the potential ATP yield from glycolytic flux from glucose increased by 340% (glucose uptake of 175 versus 603 μmol/g dry wt/150 min for control group versus G+I group) from an average approximate rate of 2.3 in the control group to 8.0 μmol ATP from glucose/g dry wt/min in the G+I group. The hearts had a wet weight/dry weight ratio of 4.83±0.07, with no significant difference between groups; thus, in protocol 2, the control group, in which contracture occurred, produced approximately 0.5 μmol glycolytic ATP from glucose/g wet wt/min, and our G+I group, in which no contracture occurred during underperfusion, produced approximately 1.7 μmol/glycolytic ATP from glucose/g wet wt/min. These results are consistent with those of Owen et al.19 who reported that “ATP production from glucose in excess of 2 μmol/g fresh wt/min” was required to prevent ischemic contracture.

In addition to preventing contracture, the increased glycolytic flux in the G+I group may also have contributed to the greater developed pressure during underperfusion and reperfusion in that group. The marked protection afforded by the high G+I substrate in the subgroups matched for identical oxygen consumption (and presumed ATP generation via oxidative phosphorylation) is consistent with the hypothesis that the glycolytic pathway may provide a small but critically localized pool of ATP, which may have a high turnover rate relative to pool size.31,52 Such glycolytic ATP appears to play an important role in membrane ion transport and protection against phospholipases.14–17,49 and these actions may have enhanced myocyte, endothelial cell, and vascular smooth muscle cell ionic homeostasis, thereby reducing cell swelling,53 microvascular compression, and impaired reflow with reperfusion.14,54,55 However, our results cannot distinguish between a direct effect of G+I intervention on the vasculature and/or a prevention of contracture and myocyte shortening as the mechanism(s) by which coronary resistance was protected during underperfusion.

The presence of the G+I substrate during reperfusion may have contributed importantly to better recovery of the G+I groups. Reperfusion is associated with oxygen free radical damage and cellular sodium and calcium overload.56,57 Thus any antioxidant and ionic homeostatic effects of the glycolytic pathway may be especially critical during reperfusion.21,22,46,58 Abnormalities in glucose metabolism persist in ischemic, but viable, myocardium in postinfarct patients.59,60 and G+I therapy has been reported to be beneficial in conjunction with thrombolytic therapy for acute infarction,38 consonant with a significant role for glycolysis during the reperfusion phase.

Role of Insulin

We assessed the effects of glucose and insulin in combination using an insulin level of 15 microunits/ml in the control hearts, similar to values reported in humans during acute myocardial infarction,34–36 and 250 microunits/ml in the G+I group, since this level was achieved in glucose-insulin-potassium clinical trials.37–41 Insulin doses up to 100 milliunits/ml (i.e., 400-fold higher) have been used in previous studies in isolated perfused hearts63,58,61; such levels may have a positive inotropic effect independent of an action on glycolysis.52–66

Previous studies4,61,67–69 suggest that the combination of glucose and insulin is more effective than either alone in stimulating glycolytic flux under ischemic conditions. No increase in intracellular glucose levels was found in hearts subjected to underperfusion when insulin (25 milliunits/ml) alone (i.e., without increased glucose) was added to the perfusate, suggesting that increased glucose availability is also required.61 During myocardial ischemia in the dog, glucose alone had a beneficial effect similar to glucose and insulin together, but to a lesser degree.4 When glucose uptake markedly decreased after treatment with catecholamines, glucose-insulin-potassium infusion restored glucose uptake.67 In isolated rat hearts, 15 mmol/l glucose stimulated glucose transport through the plasma membrane fourfold, but insulin increased it eightfold.68 This is important, since the rate of glycolysis is related to substrate availability.69

Role of Free Fatty Acids

High levels of free fatty acids depress myocardial contractility, inhibit glycolytic flux, and are potentially harmful during ischemia.45,70,71 However, in the presence of the G+I substrate, the inhibition of glycolysis by free fatty acids is minimal.70 Conversely, the G+I substrate may decrease myocardial free fatty acid uptake during ischemia71 and protect against the accumulation of toxic fatty acid derivatives.45,72 We added a physiological level of free fatty acid.
acid (palmitate) to the perfusate so that the G+1 substrate would have the possibility of exerting an anti-free fatty acid action. However, we cannot separate an anti-free fatty acid effect from other beneficial effects of the G+1 substrate.

Role of Residual Coronary Flow

At the onset of underperfusion, coronary blood flow was reduced to 11–20% of baseline in our study. Comparable residual and/or collateral flows have been measured in acute myocardial infarction in the dog. During the 150-minute underperfusion period, coronary resistance increased substantially in the control groups of both protocols 1 and 2 but not in the G+1 groups. Improved coronary perfusion should increase the benefits of the G+1 intervention, since a greater tissue washout reduces tissue lactate accumulation, tissue acidosis, and inhibition of glycolysis. There are several mechanisms by which the G+1 intervention could have prevented an increase in coronary resistance during underperfusion, such as prevention of vascular compression from myocyte diastolic shortening due to rigor or calcium overload. Additionally, the G+1 intervention may have had a direct protective effect on the vasculature itself by supporting smooth muscle plasma membrane uptake and/or increasing microvessel ATP content. A vascular protective effect of the G+1 intervention may also be important during reperfusion (see Figure 5), because myocardial flow has been observed to progressively decrease after reperfusion of a coronary occlusion.

Possible Osmotic Effect of the G+1 Intervention

It is unlikely that the higher osmolarity of the G+1 perfusate was responsible for its beneficial effects. In previous studies, substitution by a nonmetabolizable sugar of equal osmolarity for an increased glucose content did not confer the beneficial effects associated with the increased glucose level. It is also unlikely that the increase in osmolarity of the G+1 group could have accounted for the observed effects on lactate production, ATP, creatine phosphate, and glycolen.

Clinical Implications

Despite numerous previous studies (see References 4–8, 18–21, 37–41, 45, 46, 48, 74, 79, and 80) demonstrating a protective effect of increased glucose availability on hypoxic or ischemic myocardium, clinical use of this intervention has been limited. Reduced enthusiasm for the clinical use of high glucose and insulin during myocardial ischemia has been partly the result of reports concluding that glycolytic end products, such as lactic acid, may contribute to ischemic injury. Some investigators have recently concluded that a reduction of glycolysis should be beneficial to ischemic myocardium at perfusion levels that occur clinically and that glycolytic stimulation would be expected to accelerate tissue damage as a result of lactate accumulation. Our studies refute this conclusion and demonstrate a net beneficial effect of the high G+1 intervention in an experimental model that simulates the degree of underperfusion and oxygenation in an acute infarct region.

The high G+1 substrate in our study markedly protected the severely underperfused rabbit myocardium for 21/2 hours. In rabbits, the rate of ischemic necrosis is approximately four to six times more rapid than that in dogs. Therefore, high G+1 intervention may have the potential to protect ischemic myocardium in humans for a considerable period of time, perhaps 10 hours or longer, depending on the amount of collateral flow in an ischemic region. In addition to a protective effect on the ischemic myocyte, our study indicates that the G+1 substrate protects the ischemic coronary vasculature, improving its ability to maintain a low coronary resistance during prolonged low-flow underperfusion and reperfusion. Coronary resistance increases may be important in determining the amount of residual flow that persists in severely ischemic myocardium distal to a coronary occlusion and in reperfused myocardium. Thus, our results provide basic support for the use of G+1 intervention in combination with reperfusion for treatment of acute myocardial infarction.

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