Mechanisms of Glycolytic Inhibition in Ischemic Rat Hearts

By Michael J. Rovetto, William F. Lamberton, and James R. Neely

ABSTRACT
The mechanisms of glycolytic inhibition in ischemic myocardium were investigated in the isolated, perfused rat heart. Glycolysis was inhibited at the level of glyceraldehyde-3-phosphate dehydrogenase. The major factors that accounted for the glycolytic inhibition in the ischemic heart compared with the anoxic heart appeared to be higher tissue levels of lactate and H+ in the ischemic tissue. Increased extracellular pH inhibited glycolysis in anoxic and hypoxic hearts much more readily than it did in aerobic hearts. However, maintenance of both extracellular and intracellular pH caused only a modest acceleration of glycolysis in ischemic hearts. Accumulation of tissue lactate and inhibition of glycolysis were directly proportional to the reduction in coronary blood flow in both anoxic and ischemic hearts. At intracellular lactate concentrations between 15 and 20 mM, glycolysis was inhibited under both conditions. Addition of either 10, 20, or 40 mM lactate to the perfusate inhibited glycolysis in aerobic, anoxic, and ischemic hearts. The effect of lactate did not appear to be mediated through changes in intracellular pH. It is concluded that accumulation of lactate represents a major factor in the inhibition of glycolysis that develops in ischemic hearts.

Regulation of glycolysis in cardiac muscle involves control of several glycolytic enzymes (1). In aerobic tissue, glucose transport is the rate-limiting step for utilization of exogenous glucose in the absence of insulin (2), and phosphofructokinase limits utilization in the presence of insulin (3). The activity of phosphofructokinase is normally restrained by high tissue levels of inhibitors (adenosine triphosphate [ATP], creatine phosphate, and citrate) and low levels of activators such as inorganic phosphate, adenosine monophosphate (AMP), adenosine diphosphate (ADP), and fructose-1, 6-diphosphate (4, 5). Phosphorylase represents the rate-limiting step for glycogen utilization. The rate of this reaction is restrained by the low ratio of the a form of the enzyme to the b form and by inhibition of the b form by high levels of inhibitors (ATP and glucose-6-P) and low levels of the activator (5'-AMP) and the substrate (inorganic phosphate) (6).

In anoxic tissue, glucose transport is accelerated (7, 8) by as yet unknown mechanisms. Acceleration of glycogenolysis involves both a conversion of phosphorylase b to phosphorylase a and an activation of phosphorylase b by decreased intracellular levels of ATP and glucose-6-P and increased levels of 5'-AMP and inorganic phosphate (9, 10). Acceleration of phosphofructokinase results from decreased levels of ATP and creatine phosphate and increased levels of inorganic phosphate, 5'-AMP, ADP, and fructose-1,6-P (11, 12). Glycolytic flux may be accelerated several fold as a result of these changes in tissue metabolites.

Control of glycolysis in ischemic tissue is poorly understood. Most studies on regulation of glycolysis have been performed in the first few minutes after exposure of the heart to circulatory arrest (13-16). Activation of phosphorylase occurs within seconds due to increased levels of inorganic phosphate and later through conversion of the enzyme from the b to the a form and activation of the b form by decreased levels of ATP and increased levels of 5'-AMP (13). The action of phosphofructokinase appears to be the major rate-limiting step in glycolysis during the first minute (13, 16). Control of the pathway in the first 4 minutes appears to oscillate between phosphofructokinase, glyceraldehyde-3-P dehydrogenase, and pyruvate kinase (16). Longer term control of glycolysis in ischemic tissue that is not completely deprived of coronary blood flow has not been characterized. This condition may either accelerate or inhibit glycolysis depending on the rate of coronary blood flow (17, 18). Utilization of exogenous glucose is inhibited in isolated rat hearts receiving approximately 10% of the normal coronary blood flow, but it is accelerated when coronary blood flow is increased to 20 and 40% of the control rate. The lower rates of glycolysis in ischemic tissue occur in spite of changes in the tissue levels of adenine
nucleotides, creatine phosphate, and inorganic phosphate that are similar to those found in anoxic tissue (9). The purpose of the present study was to determine the mechanism(s) of glycolytic restraint in ischemic hearts at several rates of coronary blood flow.

**Methods**

HEART PERFUSION

Hearts were removed from 200-250-g male Sprague-Dawley rats and perfused by either the working heart technique or the Langendorff procedure as described previously (20). The perfusate consisted of Krebs-Henseleit-bicarbonate buffer containing the substrates shown in the figures and tables. The perfusate was bubbled with a 95% O₂-5% CO₂ gas mixture for control hearts, a 95% N₂-5% CO₂ gas mixture for anoxic hearts, and a 2% O₂-75% N₂-5% CO₂ gas mixture for hypoxic hearts. Anoxic and hypoxic hearts were perfused by the Langendorff procedure, and coronary blood flow was maintained with a hydrostatic aortic perfusion pressure. Control working hearts were perfused with a constant left atrial filling pressure of 7.5 mm Hg and a hydrostatic aortic afterload of 60 mm Hg. Whole-heart ischemia was induced in the working hearts by placing a one-way valve in the aortic outflow tract as described previously (19). This valve did not interfere with ventricular ejection of the perfusate but prevented retrograde perfusion of the coronary arteries during diastole. As a result of a lower aortic perfusion pressure during diastole, coronary blood flow initially decreased by 60% from the control level and continued to decline during ischemic perfusion as ventricular failure progressed. The rate of flow was maintained at various levels after ventricular failure occurred (18) by providing a minimum aortic perfusion pressure.

**MEASUREMENT OF GLYCOLYTIC RATES AND TISSUE METABOLITES**

Utilization of exogenous glucose was estimated by measuring the rate of ²H₂O production from either 2-hydroxy- or 5-hydroxy-glucose as described previously (21). Tritium in either the 2 or the 5 position of glucose is exchanged with H₂O in the glycolytic reactions. The rate of ²H₂O production was estimated by collecting samples of coronary effluent at various times during the perfusion, separating the labeled glucose from ²H₂O, and counting the H₂O sample in a Beckman LS-230 liquid scintillation counter. Separation of ²H₂O from ²H-glucose was achieved by placing a 0.2 ml sample of perfusate on a 0.5 x 2-cm column of Dowex-1 borate (22, 23), which retained labeled glucose. The sample of ²H₂O was washed from the column directly into a counting vial with 0.8 ml of H₂O₂. At the end of the perfusion, the hearts were quick frozen by clamping them between Wollenberger clamps cooled in liquid nitrogen. The tissues were powdered in a percussion mortar maintained at the temperature of liquid nitrogen and extracted in ice-cold 6% perchloric acid. Tissue levels of glycolytic intermediates, adenine nucleotides, and creatine phosphate were measured in the neutralized perchloric acid extract by standard enzymatic procedures (24) using either a spectrophotometer or a fluorometer. Extracellular pH was estimated by measuring the pH of the coronary venous effluent which was collected under mineral oil without exposure to air and maintained at 37°C. Intracellular pH was calculated from the tissue distribution of ¹⁴C-5,5-dimethyl-2,4-oxazolidinedione (DMO) (25); 5-³H-sorbitol was used as an extracellular marker.

**Results**

**SITE OF GLYCOLYTIC INHIBITION IN ISCHEMIC HEARTS**

Data presented in a previous paper (18) indicate that glycolysis is restrained in ischemic hearts at all rates of coronary blood flow compared with that in anoxic tissue. This restraint does not appear to result from lack of substrate delivery to the cells, since only a small fraction (approximately 10%) of the arterial glucose is removed by the heart and intracellular levels of glucose-6-P increase (17). To determine the site of glycolytic inhibition, tissue levels of glycolytic intermediates were measured at several times after the induction of ischemia. The levels of intermediates in control and ischemic hearts after 16 minutes of perfusion are shown in Table 1. These data and the levels of intermediates measured after 2 and 8 minutes of ischemia are also shown in Table 1. The perfusate contained 11 mM glucose and was bubbled with a 95% O₂-5% CO₂ gas mixture. Hearts received a 10-minute washout perfusion at 60 mm Hg of aortic pressure, a 10-minute perfusion under either control working or ischemic conditions. A minimum aortic perfusion pressure was not provided in the ischemic hearts, and coronary blood flow declined progressively as ventricular failure occurred, averaging about 0.6 ml/min after 16 minutes. Coronary blood flow in the control hearts averaged 15 ml/min. The values represent the means ± S.E. for ten hearts under each condition. G6P = glucose-6-P, F6P = fructose-6-P, FDP = fructose-1,6-diphosphate, G3P = glyceraldehyde-3-P, DHP = dihydroxyacetone phosphate, 3PG = 3-phosphoglycerate, 2PG = 2-phosphoglycerate, PEP = phosphoenolpyruvate, Pyr = pyruvate, Lac = lactate, and aGP = α-glycerol-P.

<table>
<thead>
<tr>
<th>Glycolytic intermediate</th>
<th>Control (nmoles/g dry weight)</th>
<th>Ischemic (nmoles/g dry weight)</th>
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<tbody>
<tr>
<td>G6P</td>
<td>456 ± 52</td>
<td>1300 ± 130</td>
</tr>
<tr>
<td>F6P</td>
<td>100 ± 11</td>
<td>226 ± 36</td>
</tr>
<tr>
<td>FDP</td>
<td>190 ± 19</td>
<td>510 ± 33</td>
</tr>
<tr>
<td>G3P</td>
<td>80 ± 10</td>
<td>209 ± 39</td>
</tr>
<tr>
<td>DHP</td>
<td>69 ± 11</td>
<td>581 ± 43</td>
</tr>
<tr>
<td>3PG</td>
<td>183 ± 15</td>
<td>222 ± 23</td>
</tr>
<tr>
<td>2PG</td>
<td>46 ± 8</td>
<td>64 ± 6</td>
</tr>
<tr>
<td>PEP</td>
<td>49 ± 10</td>
<td>68 ± 9</td>
</tr>
<tr>
<td>Pyr</td>
<td>199 ± 15</td>
<td>152 ± 13</td>
</tr>
<tr>
<td>Lac</td>
<td>5400 ± 1700</td>
<td>47,000 ± 6200</td>
</tr>
<tr>
<td>αGP</td>
<td>269 ± 53</td>
<td>6500 ± 240</td>
</tr>
</tbody>
</table>

The perfusate contained 11 mM glucose and was bubbled with a 95% O₂-5% CO₂ gas mixture. Hearts received a 10-minute washout perfusion at 60 mm Hg of aortic pressure, a 10-minute perfusion as control working hearts, and an additional 16-minute perfusion under either control working or ischemic conditions. A minimum aortic perfusion pressure was not provided in the ischemic hearts, and coronary blood flow declined progressively as ventricular failure occurred, averaging about 0.6 ml/min after 16 minutes. Coronary blood flow in the control hearts averaged 15 ml/min. The values represent the means ± S.E. for ten hearts under each condition. G6P = glucose-6-P, F6P = fructose-6-P, FDP = fructose-1,6-diphosphate, G3P = glyceraldehyde-3-P, DHP = dihydroxyacetone phosphate, 3PG = 3-phosphoglycerate, 2PG = 2-phosphoglycerate, PEP = phosphoenolpyruvate, Pyr = pyruvate, Lac = lactate, and αGP = α-glycerol-P.
Effects of ischemia on tissue levels of glycolytic intermediates. Hearts were perfused as described for Table 1. Coronary blood flow averaged 15 ml/min in control hearts and about 5, 3, and 0.6 ml/min in ischemic hearts after 2, 8, and 16 minutes of perfusion, respectively. The metabolite levels in ischemic hearts are given as percents of the control levels. Each value represents the mean of at least ten determinations. G6P = glucose-6-P, F1,6-P = fructose-1,6-diphosphate, G3P = glyceraldehyde-3-P, 2PG = 2-phosphoglycerate, Pyr = pyruvate, aGP = α-glycerol-P, F6P = fructose-6-P, DHP = dihydroxyacetone phosphate, 3PG = 3-phosphoglycerate, PEP = phosphoenolpyruvate and Lact = lactate.

shown in Figure 1 where the values for ischemic hearts are expressed as percents of the control levels. At the time the tissues were frozen for analysis of intermediates, coronary blood flow averaged about 15 ml/min for control hearts and 5.3, 3.0, and 0.6 ml/min for ischemic hearts after 2, 8, and 16 minutes, respectively. The rate of glucose utilization declined from about 6 to 3 μmoles/g dry weight min⁻¹ during the course of ischemic perfusion.

The tissue levels of hexose phosphates (glucose-6-P, fructose-6-P, and fructose-1, 6-P) were elevated above the control levels after 2 minutes of ischemia and remained high throughout the perfusion. Levels of both lactate and α-glycerol phosphate increased in proportion to the reduction in coronary blood flow as perfusion was continued. One of the most striking changes occurred in the levels of triose phosphates. The level of dihydroxyacetone-P (DHP) increased much more than did the level of glyceraldehyde-3-P (G3P). The DHP-G3P ratio was 0.86 in control hearts and 3.67, 2.72, and 2.78 in ischemic hearts after 2, 8, and 16 minutes, respectively. This large increase in triose phosphates associated with the reduction in glycolytic flux indicates that the rate of glyceraldehyde-3-P dehydrogenase limits glycolysis in ischemic tissue.

Changes in the tissue levels of glycolytic intermediates during ischemia in hearts perfused with high levels of glucose and insulin are shown in Figure 2. In this case, tissue levels of hexose phosphates were higher in control hearts than they were when insulin was not present (1580 ± 90, 359 ± 30, and 278 ± 31 nmole/g dry weight for glucose-6-P, fructose-6-P, fructose-1,6-P, respectively), and the major increases during ischemia occurred in the levels of triose phosphates and fructose-1,6-P. These changes in intermediates were associated with an inhibition of glycolysis similar to that in the noninsulin-treated hearts (17) and again indicate inhibition of glyceraldehyde-3-P dehydrogenase.

The rise in the levels of triose phosphates could simply reflect a mass action effect due to an increase in the products of the glyceraldehyde-3-P dehydrogenase reaction and, therefore, would not indicate that the reaction was rate limiting. Both products of the enzyme, NADH and 1,3-diphosphoglycerate are difficult to accurately measure in tissue extracts. However, since the level of 3-phosphoglycerate did not change appreciably, it is unlikely that the level of 1,3-diphosphoglycerate was altered significantly. The other product of the reaction (NADH) increased as indicated by the lactate-pyruvate ratio, which increased from 27 in control hearts to 54, 250, and 493 in ischemic hearts.
after 2, 8, and 16 minutes, respectively. The \( \alpha \)-glycerol phosphate-dihydroxyacetone phosphate ratio increased from 3.9 in control hearts to 5.8, 9.6, and 11.2 in ischemic hearts after 2, 8, and 16 minutes, respectively. The NADH-NAD ratio as calculated from the lactate-pyruvate ratio and the measured intracellular pH, using 1.11 \( \times \) 10\(^{-11} \) as the equilibrium constant for lactate dehydrogenase (26), increased from 3.2 in control hearts to 5.5, 20.5, and 36.9 \( \times \) 10\(^{-10} \) in ischemic hearts after 2, 8, and 16 minutes, respectively.

The coupled reactions, 3-P-glycerate kinase and glyceraldehyde-3-P dehydrogenase, remain in equilibrium in liver under a variety of metabolic conditions (27). Since it is difficult to measure the equilibrium in liver under a variety of metabolic conditions (27). Since it is difficult to measure the equilibrium in liver under a variety of metabolic conditions (27). Since it is difficult to measure the equilibrium in liver under a variety of metabolic conditions (27). Since it is difficult to measure the equilibrium in liver under a variety of metabolic conditions (27). Since it is difficult to measure the equilibrium in liver under a variety of metabolic conditions (27).

The rise in NADH may have contributed to the reduced flux through glyceraldehyde-3-P dehydrogenase under ischemic conditions, but since the NADH-NAD ratio was used in calculating the mass action ratios for the coupled reactions, high NADH would not appear to be the only factor accounting for glycolytic inhibition. Also, the NADH-NAD ratio increases in anoxic tissue (12) in which glycolysis is accelerated. Therefore, factors other than high NADH may have limited glycolysis in ischemic hearts.

### EFFECTS OF EXTRACELLULAR pH ON GLYCOLYSIS IN AEROBIC, HYPOXIC, ANOXIC, AND ISCHEMIC HEARTS

The major difference between ischemic and anoxic hearts is the rate of coronary blood flow. Both conditions result in oxygen deficiency and in similar levels of adenine nucleotides, creatine phosphate, and inorganic phosphate (activators and inhibitors of glycolysis). In association with sluggish coronary blood flow in ischemic hearts, however, levels of tissue lactate are much higher than they are in anoxic hearts (17). In addition, both extracellular and intracellular pH decrease to a larger extent in ischemic hearts than they do in anoxic hearts (18). Therefore, accumulation of lactate and decreased tissue pH appear to be major factors that could account for inhibition of glycolysis in ischemic hearts, and the influence of these factors on glucose utilization was determined.

### TABLE 2

<table>
<thead>
<tr>
<th>CO(_2) (%)</th>
<th>Arterial perfusate</th>
<th>Coronary venous effluent</th>
<th>Intracellular pH</th>
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<tbody>
<tr>
<td>30</td>
<td>6.55</td>
<td>6.55</td>
<td>6.58 ± 0.05</td>
</tr>
<tr>
<td>30</td>
<td>6.70</td>
<td>6.70</td>
<td>6.70 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>7.05</td>
<td>7.00</td>
<td>6.88 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>7.35</td>
<td>7.25</td>
<td>6.96 ± 0.02</td>
</tr>
<tr>
<td>1.8</td>
<td>7.74</td>
<td>7.46</td>
<td>6.98 ± 0.02</td>
</tr>
</tbody>
</table>

In aerobic hearts perfused with buffer at pH 7.35, the coronary effluent pH was 7.25 and the intracellular pH was 7.0. When the coronary effluent pH was about 7.0 or below, intracellular and extracellular levels of pH were about equal, indicating that intracellular pH can be altered by varying extracellular pH (Table 2). The effects of altering extracellular pH under aerobic, hypoxic, and anoxic conditions are shown in Figure 3. The rate of coronary blood flow was maintained at about 15 ml/min in each case. In aerobic hearts, rates of glycolysis were unaffected when the perfusate pH was decreased to 6.75, a level which reduces intracellular pH to 6.70. This pH is comparable to the intracellular pH measured in ischemic hearts in which glycolysis is inhibited (18). Glycolysis was significantly reduced, however, when the pH was decreased to 6.55. The rate of glycolysis was more sensitive to inhibition by lowering the extracellular pH in hypoxic and anoxic hearts. A pH of 6.80 prevented the hypoxic acceleration of glycolysis, and a pH of either 7.00 or 6.75 produced significant reductions in the accelerated rate of glycolysis in anoxic hearts. The anoxic rate could not be increased further by raising the perfusate pH to 7.70. These data indicate that glycolysis in oxygen-deficient hearts is more susceptible to inhibition by low pH and suggest that a combination of low pH and either a high NADH-NAD ratio or higher tissue levels of lactate may effectively inhibit glycolysis in ischemic hearts.

In the preceding experiments, coronary blood flow was maintained at about 15 ml/min in each
Effects of extracellular pH on glycolysis in aerobic, hypoxic and anoxic hearts. The perfusate contained 11 mM glucose. During the first 10 minutes of perfusion in aerobic and hypoxic hearts, the perfusate was bubbled with a 95% O₂-5% CO₂ gas mixture and in anoxic hearts with a 95% N₂-5% CO₂ gas mixture. The perfusate pH was 7.35 under these conditions. Aerobic hearts were perfused in the working heart apparatus (20) with a left atrial pressure of 10 cm H₂O. Hypoxic and anoxic hearts were perfused as Langendorff preparations with aortic perfusion for a pH of 7.35 or switched to a perfusate bubbled with 98% O₂ for a pH of 7.70. In anoxic hearts, perfusion was either continued at pH 7.35 or switched to a buffer adjusted to pH 6.75 or 6.55 by bubbling with a gas mixture of 70% O₂-30% CO₂ and adjusting the pH with HCl. Under these conditions, the intracellular pH as determined by the DMO procedure was 7.01 ± 0.01, 6.70 ± 0.03, and 6.58 ± 0.05, respectively. Hypoxia was induced after 10 minutes by switching to a perfusate bubbled with 75% N₂, 20% O₂, 5% CO₂ for a pH of 7.35 or 50% N₂, 20% O₂, 30% CO₂ for a pH of 6.80. In anoxic hearts, perfusion was either continued at a pH of 7.35 or switched to a perfusate bubbled with 98% N₂, 1.8% CO₂ for a pH of 7.7, 90% N₂, 10% CO₂ for a pH of 7.0, or 70% N₂, 30% CO₂ for a pH of 6.75. Each point represents the mean ± SE for 6-12 hearts.

Effects of perfusate buffer capacity on glycolysis in ischemic hearts. Hearts were perfused under ischemic conditions with either regular Krebs-Henseleit-bicarbonate buffer (solid lines) or bicarbonate-buffer fortified with Hepes (open circles) at either 25 mM (A) or 50 mM (B) and with 50 mM Tricine (solid triangles, B). In each case, the concentration of NaCl was decreased to compensate for the extra osmolarity caused by addition of the buffers. Tissue levels of glycolytic intermediates were measured at the end of 15 minutes (A) and 20 minutes (B) and are expressed as the percent change in those hearts receiving fortified buffer compared with ischemic hearts receiving regular bicarbonate buffer. Glucose was present at 11 mM in each case, and ischemia was induced at zero time. In A, no attempt was made to maintain coronary blood flow as ventricular failure occurred, and after 15 minutes, flow rates were 0.6 ± 0.1 and 1.2 ± 0.2 ml/min for regular and Hepes buffers, respectively. In B, a minimum constant aortic perfusion pressure of 30 cm H₂O was provided after ventricular failure occurred, and flow rates averaged 2.35 ± 0.25, 4.6 ± 0.3, and 4.2 ± 0.3 ml/min in regular bicarbonate, Hepes, and Tricine buffers, respectively. The coronary effluent pH was 7.0 with bicarbonate alone or 7.4 with the other buffers. Each value represents the mean ± SE for six hearts.
Mechanisms of Glycolytic Inhibition

Heart's receiving regular bicarbonate buffer showed the usual decline in ventricular performance and coronary blood flow during the course of ischemic perfusion (17, 19), and glycolysis was inhibited. Coronary blood flow decreased from 6 ml/min initially to 0.6 ml/min after 15 minutes. Hearts receiving the fortified buffer showed a similar pattern of decline in mechanical performance, but the decline in coronary blood flow was somewhat less (to 1.2 ml/min after 15 minutes), and the rate of glycolysis did not decrease. This maintenance of the glycolytic rate was associated with small decreases in glucose-6-P, fructose-1,6-P, and triose phosphates and with increased levels of phosphoenolpyruvate and pyruvate. These changes may indicate an improved flux through glyceraldehyde-3-P dehydrogenase.

In figure 4B, the perfusate contained either regular buffer, 50 mM Hepes, or 50 mM Tricine at pH 7.4. Ischemia was induced at zero time, and glycolysis was measured at the times indicated. In this case, a minimum aortic perfusion pressure of 30 cm H₂O was provided to maintain coronary blood flow. The rate of flow declined from 6 ml/min at zero time to about 2.4, 4.6, and 4.2 ml/min after 20 minutes in hearts receiving regular bicarbonate, Hepes, and Tricine buffers, respectively. The rate of glucose utilization increased somewhat during the course of perfusion with bicarbonate buffer due to the maintenance of a minimum coronary blood flow (18) and was significantly accelerated when either Hepes or Tricine was present. This acceleration of glycolysis was associated with a 50% decrease in the levels of glucose-6-P and small decreases in pyruvate and lactate. The tissue levels of glucose-6-P were 1.35 ± 0.05, 0.59 ± 0.03, and 0.70 ± 0.04 nmoles/g dry weight in the bicarbonate-, Hepes-, and Tricine-perfused hearts, respectively. These data suggest that flux through the phosphofructokinase reaction was stimulated by the extra buffering capacity, the associated improvement in coronary blood flow, or both.

Ventricular performance was not improved by the additional buffer, but coronary vascular resistance decreased. The same aortic perfusion pressure resulted in about twice the coronary blood flow in these hearts compared with that in hearts perfused with bicarbonate buffer alone. This improvement in coronary blood flow, rather than direct buffering of the intracellular compartment, may have been responsible for most of the increase in glycolysis (Fig. 4).

Since the rate of glycolysis is proportional to coronary blood flow in ischemic tissue (18), it was of interest to determine the effects of coronary blood flow on glycolytic intermediates. Intermediates were measured after 20 minutes of perfusion and the levels in hearts receiving a coronary blood flow of 2.3 and 4 ml/min are expressed as a percent of those in hearts receiving a coronary flow of 0.6 ml/min (Fig. 5). Increasing coronary blood flow improved glycolytic rates and was associated with reduced tissue levels of hexose phosphates and triose phosphates and increased levels of pyruvate, indicating that flux through glyceraldehyde-3-P dehydrogenase was accelerated. Increasing the rate of coronary blood flow also reduced the tissue levels of lactate and increased pH (18).

The results presented in Figures 4 and 5 indicate that improved coronary blood flow accelerates glycolysis in ischemic tissue by relieving the restraint on glyceraldehyde-3-P dehydrogenase.

Effects of coronary blood flow on glycolysis and glycolytic intermediates. Hearts were perfused with buffer equilibrated with a 95% O₂-5% CO₂ gas mixture; the perfusate contained 11 mM glucose. Ischemia was induced at zero time, and in some hearts coronary blood flow was allowed to decline to 0.6 ml/min as ventricular failure occurred (shown as 100% line for intermediates). In others, flow was maintained at either 2.3 ml/min (solid circles) or 4.0 ml/min (open circles). Glycolytic intermediates were measured after 20 minutes of ischemia. The levels in hearts receiving 2.3 and 4.0 ml/min of coronary blood flow are presented as percents of the levels in hearts receiving 0.6 ml/min. Abbreviations are the same as they are in Figure 1. Absolute levels of intermediates at 0.6 ml/min of coronary blood flow were similar to those in Table 1 after 16 minutes of ischemia. Each value represents the mean for six hearts.

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Most of the increase in glycolysis that occurred when the perfusate contained additional buffers (Fig. 4B) was probably due to the increase in coronary blood flow. However, the glycolytic rates with 50 mM Hepes or with Tricine when coronary blood flow was increased to about 4 ml/min were somewhat greater than those achieved by maintaining coronary blood flow at 4 ml/min without additional buffer (Fig. 5). Also, the changes in intermediates indicate that the extra buffer may have an additional effect by accelerating phosphofructokinase (indicated in Fig. 4B by the decreased levels of glucose-6-P) as well as by improving coronary blood flow.

EFFECTS OF TISSUE LACTATE ON GLYCOLYSIS

Inhibition of glycolysis was consistently associated with increased tissue levels of lactate. The relationship between glycolytic rate and tissue lactate in ischemic and anoxic hearts is illustrated in Figure 6. In both cases, perfusate containing no lactate was allowed to pass through the heart only a single time, and tissue levels of lactate were increased by restricting coronary blood flow. In hearts receiving perfusate bubbled with 95% O₂-5% CO₂, glycolysis was accelerated when the tissue was made hypoxic by reducing coronary blood flow to about 5 ml/min, and intracellular lactate increased to about 10 mM. With further reductions in coronary blood flow, tissue lactate continued to accumulate, and glycolysis was inhibited. The data from Figure 4 for hearts perfused with extra buffers in the perfusate are shown by open circles for comparison. These values indicate that for a given level of tissue lactate glycolytic flux was only modestly improved and that at high levels of lactate (32 mM) inhibition of glycolysis still developed. In the anoxic hearts, glycolysis was accelerated when coronary blood flow was maintained at 15 ml/min and lactate accumulation was low. However, when coronary blood flow was restricted to 2 ml/min and lactate accumulated to 20 mM, glycolytic inhibition occurred. These data indicate an inverse relationship between tissue lactate and glycolysis.

The effects of adding lactate to the perfusate of anoxic and ischemic hearts are shown in Figure 7. In the anoxic hearts, coronary blood flow was maintained at 15 ml/min. Tissue lactate increased to about 8 mM in those hearts perfused without added lactate and to about 30 mM when 20 mM lactate was included in the perfusate. The addition of lactate prevented anoxic acceleration of glycolysis. In hearts perfused at control rates of coronary blood flow with well-oxygenated perfusate, addition of 40 mM lactate effectively inhibited glycolysis (zero time for ischemic hearts in Fig. 7). Tissue lactate, at this time, was 3 and 40 mM in hearts perfused with and without lactate, respectively. In the ischemic hearts in which coronary blood flow was maintained at 4 ml/min, addition of lactate prevented acceleration of glycolysis. Intracellular lactate increased to 10 and 60 mM in the absence and the presence of perfusate lactate, respectively. Glycolytic rates for ischemic hearts in which coronary blood flow was allowed to decline to 1.0 ml/min and intracellular lactate increased to 20 mM without added lactate are shown for comparison (broken line). Glycolysis was inhibited to about the same extent in these hearts as it was in those receiving a higher coronary blood flow but with lactate added to the buffer. Lower levels of extracellular lactate were also effective in reducing glycolysis in ischemic hearts. The addition of 10 mM sodium lactate (pH 7.4) to the perfusate in hearts receiving a coronary blood flow of 4 ml/min increased intracellular lactate from 10 to 30 mM and reduced glycolysis from 8.5 ± 0.5 to 3.0 ± 0.2 μmoles/g min⁻¹.

Discussion

In the isolated rat heart, oxygen deficiency as a result of reduced coronary blood flow leads to an initial acceleration of glycolysis due to a more rapid rate of glycogenolysis (17), an effect similar to that seen in the in situ heart when blood flow is arrested (13–16). However, glycogenolysis occurs at a slower
Effects of extracellular lactate on glycolysis in anoxic and ischemic hearts. The perfusate contained 11 mM glucose (solid circles) and lactate at either 20 mM (broken line, top section) or 40 mM (triangles, bottom section). In the anoxic hearts, coronary blood flow was maintained at about 14 ml/min. In the ischemic hearts, coronary blood flow was either maintained at 4 ml/min (solid lines) or allowed to decline to 1.0 ml/min (broken line). Each value represents the mean ± SE for six determinations.

As shown by the work of J. H. Park and his co-workers (29-31), the activity of glyceraldehyde-3-P dehydrogenase is controlled by a number of the same metabolites that regulate the activity of phosphofructokinase. Both enzymes are sensitive to changes in pH over the pH range observed in control and ischemic hearts in the present studies. ATP and creatine phosphate inhibit both enzymes, and the decrease in these high-energy phosphates in anoxic and ischemic tissue would be expected to accelerate both reactions. Inorganic phosphate accelerates both reactions, and the rise in this compound would be expected to increase glycolysis. However, the increase in tissue levels of ADP and AMP would function to activate phosphofructokinase, whereas these compounds inhibit glyceraldehyde-3-P dehydrogenase just as effectively as does ATP. In addition, glyceraldehyde-3-P dehydrogenase is much more sensitive to inhibition by adenine nucleotides at pH 6.8 than it is at pH 7.4. Therefore, the greater decrease in cellular pH in ischemic hearts compared with that in anoxic hearts would allow residual amounts of adenine nucleotides and creatine phosphate to inhibit glyceraldehyde-3-P dehydrogenase more effectively in ischemic tissue than in anoxic tissue.

From the data presented in the present study, it appears that inhibition of glycolysis in ischemic tissue results from the concerted action of several factors. A reduction in extracellular pH sufficient to lower intracellular pH to 6.8, the lowest seen in ischemic tissue, does not inhibit glycolysis in aerobic hearts but is an effective inhibitor under anoxic or hypoxic conditions. This observation indicates that both a low pH and either a high NADH level, a reduced phosphate potential, or both, effectively inhibit glycolysis. However, elevation of intracellular pH alone results in only modest improvements in glycolysis in ischemic hearts, and these improvements appear to occur at the level of
phosphofructokinase. Elevated perfusate pH does reduce coronary vascular resistance and improve coronary blood flow at a constant aortic perfusion pressure. This increase in coronary blood flow accounts for most of the acceleration of glycolysis that occurs when the buffer capacity of the perfusate is increased.

The other major factor that may account for inhibited glycolysis in ischemic tissue is the large accumulation of lactate. Lactate is known to reduce glycolysis in aerobic tissue (32), presumably by serving as an alternate substrate for oxidative metabolism. In the present study, addition of lactate to the perfusate caused a large reduction in glycolysis in aerobic, ischemic, and anoxic hearts. The fact that extracellular lactate inhibits glycolysis in oxygen-deficient tissue indicates that the effect does not depend on oxidation and suggests a role for lactate other than simply as an alternate substrate. Fatty acids, which serve as an alternate substrate and inhibit glycolysis in aerobic hearts (33, 34), do not reduce glucose utilization in ischemic hearts (18) or glucose transport in anoxic tissue (34). The mechanism of the lactate effect is unknown, but it does not appear to involve a change in intracellular pH. Anoxic hearts perfused both with and without 20 mM lactate had an intracellular pH of 7.00 ± 0.05. In ischemic hearts receiving a coronary blood flow of 3 ml/min, intracellular pH was 6.89 ± 0.03 and 6.81 ± 0.03 with and without the addition of 17 mM sodium lactate. Also, including 20 mM Tris buffer along with 10 mM lactate does not prevent glycolytic inhibition.

Since tissue lactate would be expected to accumulate in proportion to the extracellular concentration, glycolysis may be more severely depressed when coronary blood flow is restricted in vivo than it is in isolated hearts perfused with lactate-free buffer. This fact may account for the observed inhibition of glycolysis in blood-perfused swine hearts when coronary blood flow is reduced by 60% (35). It may also indicate that in the clinical development of regional ischemia glycolysis is not increased regardless of the degree of flow restriction.

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