Effects of insulin on glucose uptake by rat hearts during and after coronary flow reduction

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IT HAS PREVIOUSLY been reported (13–15) that both anoxia as well as low-flow ischemia enhance glucose uptake in the isolated, perfused heart. More recently it has also been shown that anoxia and ischemia, like insulin (35), cause translocation of the insulin-responsive glucose transporter GLUT-4 in the heart (24, 31, 34). It is likely that glucose transport is rate limiting for the subsequent metabolism of glucose, at least before stimulation of glucose transporter activity.

Although the exact mechanisms linking O₂ deprivation and glucose uptake are not known, it appears that the first prerequisite for enhanced glucose uptake is the translocation of the glucose transporter proteins (24, 34) and the second prerequisite is the removal of lactate and protons, which can inhibit glycolysis. The current hypothesis is that glycolysis is enhanced by low-flow ischemia (15) but inhibited by total ischemia (21). These experimental observations provide an explanation for clinical studies that suggest enhanced glucose uptake by ischemic, partially perfused, or reperfused heart muscle in vivo, reflected by the retention of the glucose tracer analog 2-deoxy-2-[¹³C]fluoro-d-glucose (27).

The starting point for the present work was the observation by Neely et al. (15) that in the isolated, working rat heart subjected to reversible low-flow ischemia (reduction of coronary flow by 75%), there is an increase in glucose uptake. Because insulin, like O₂ deprivation, stimulates glucose uptake in heart muscle (1, 13, 14), we were interested to learn whether low-flow ischemia alone already maximizes glucose uptake and, consequently, decreases insulin responsiveness. Alternatively, we speculated that the effects of insulin and hypoxia could be additive. We assessed the effect of insulin on glucose uptake before, during, and after the onset of a reversible reduction of coronary flow with and without competing substrates. We found that hearts retained the same responsiveness to insulin during coronary flow reduction as under control conditions.

The most pronounced effect of insulin on glucose uptake, however, was observed when insulin was added immediately after the low-flow period, at the beginning of the postischemic period. At the same time, we noted a net increase in glycogen, suggesting that glucose is rerouted from glycolysis to glycogen synthesis.

MATERIALS AND METHODS
Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–400 g were fasted overnight (16–22 h) with free access to water. We used fasted animals because their hearts show greater insulin responsiveness than hearts from fed animals. The use of animals and the experimental protocol were approved by the Animal Welfare Committee of the University of Texas Houston Health Science Center.

Materials. All chemicals were of analytical grade and were obtained from Fisher Scientific (Lexington, MA) or Sigma Chemical (St. Louis, MO). Enzymes and cofactors were obtained from Sigma Chemical or Boehringer Mannheim (Indianapolis, IN). D-[2-³H]glucose was purchased from DuPont NEN (Boston, MA).

Heart perfusions and perfusion protocol. Hearts were perfused as working hearts in the apparatus described earlier (25). Briefly, rats were anesthetized with pentobarbital sodium (100 mg/kg), and heparin (200 U) was administered via the inferior vena cava. Hearts were rapidly excised and placed in ice-cold Krebs-Henseleit buffer. After the aorta was cannulated, retrograde (Langendorff) perfusion was begun with oxygenated Krebs-Henseleit buffer (equilibrated with 95% O₂–5% CO₂, 37°C) containing 10 mM glucose and 2.5 mM CaCl₂ until the left atrium was cannulated and any blood remaining in the heart was washed out. Hearts were then switched to a working mode with recirculating Krebs-Henseleit buffer (200 ml) containing glucose (5 or 10 mM),...
glucose (10 mM) plus l-lactate (5 mM; sodium salt), glucose (10 mM) plus octanoate (5 mM; sodium salt), or glucose (10 mM) plus D,L-β-hydroxybutyrate (BOH; 10 mM; sodium salt).

The perfusion protocol (Fig. 1) consisted of a period of perfusion at near-physiological workload (100 cmH₂O afterload, 15 cmH₂O preload) for 15 min, followed by 45 min of low-flow ischemia induced by lowering the afterload (coronary perfusion pressure) to 35 cmH₂O while maintaining the same preload (15 cmH₂O). For the final 30 min of perfusion, the afterload was returned to 100 cmH₂O. We defined this as the postischemic period. Bolukoglu et al. (2) and Taegtmeyer et al. (26) had previously shown a linear relation between oxygen consumption and metabolic rate of glucose and lactate. The accumulation of metabolites.

Because cardiac power for groups with glucose (G) alone (G5, G10 groups, respectively), glucose (10 mM) plus BOH (10 mM; B groups), glucose (10 mM) plus lactate (5 mM; L groups), glucose (10 mM) plus octanoate (5 mM; O groups), or glucose (10 mM) plus BOH (10 mM; B groups). Octanoate was investigated as a cosubstrate to simulate the effect of rapid fatty acid oxidation, because this short-chain fatty acid bypasses carnitine palmitoyl transferase, which limits the access of long-chain fatty acids to β-oxidation. Lactate and BOH were chosen as second substrates because these are physiological oxidizable substrates for the heart, and lactate is the glycolytic end product. Insulin (1 mU/ml) was added at 0, 30, or 60 min and remained present to end of experiment. D,L-BOH, D,L-β-hydroxybutyrate.

We studied twelve groups of hearts. The designations of the groups are given in Table 1. Substrates were glucose (5 or 10 mM) alone (G5 and G10 groups, respectively), glucose (10 mM) plus D,L-β-hydroxybutyrate (BOH; 10 mM; sodium salt). Values are means ± SD for 3–15 observations. L, lactate; B, β-hydroxybutyrate; O, octanoate.

until insulin was added to experiments, perfusion conditions were identical. Values are means ± SD for 3–15 observations. L, lactate; B, β-hydroxybutyrate; O, octanoate.

We use the term low-flow ischemia to distinguish our model from the more commonly used model of no-flow ischemia, although we are aware that, excepting O₂ delivery, the degree of ischemia still permits adequate delivery of substrates and removal of metabolites.

The average coronary flow was 16 ml·min⁻¹·g wet wt⁻¹ before and after ischemia and 4.7 ml·min⁻¹·g wet wt⁻¹ during low-flow ischemia (70% reduction in coronary flow). We studied twelve groups of hearts. The designations of the groups are given in Table 1. Substrates were glucose (5 or 10 mM) alone (G5 and G10 groups, respectively), glucose (10 mM) plus BOH (10 mM; sodium salt). Values are means ± SD for 3–15 observations. L, lactate; B, β-hydroxybutyrate; O, octanoate.

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Springs, OH). Rates of glucose uptake and lactate release (µmol·min⁻¹·g dry wt⁻¹) were calculated by linear regression analysis of the relationship of ³H₂O or lactate content of the perfusate over time during the three time periods of the perfusion: physiological coronary flow, reduced coronary flow, and return to physiological coronary flow.

Tissue metabolites. Freeze-clamped hearts were ground to a fine powder under liquid N₂ and extracted with ice-cold 6% perchloric acid. After the extract was neutralized with KOH, the citrate level was determined using a standard enzymatic assay. Glycogen was extracted using an ethanol precipitation of tissue digested in hot 30% KOH and measured as glucose after digestion with amyloglucosidase (30). A sample of the frozen tissue powder was used for wet and dry weight determinations. Tissue metabolites are reported as nanomoles per gram of dry weight, except for glycogen, which is reported as micromoles of glycosyl units per gram of dry weight.

Statistical analysis. Data are presented as means ± SD. Statistical comparisons were performed using a single-factor analysis of variance and post hoc comparisons by Tukey's test. Differences were considered statistically significant when P < 0.05.

RESULTS

Contractile performance. Figure 1 shows the perfusion protocol and contractile performance (cardiac power), depicting in descending order hearts perfused with glucose alone, glucose plus lactate, glucose plus BOH, or glucose plus octanoate. Individual groups (see Table 1) are represented by symbols that refer to perfusion without insulin addition or with insulin addition at 30 or 60 min (arrows). Because cardiac performance of groups G₅, G₅-I-30, and G₁₀-I-0 did not differ from the other groups, data were omitted for clarity. Neither the addition of insulin nor the presence of a second exogenous substrate affected contractile function. During low-flow ischemia, coronary flow decreased from 16 ± 2 to 4.7 ± 0.4 ml·min⁻¹·g wet wt⁻¹ (P < 0.001). Cardiac power decreased in all groups by 75 ± 8%. Both values returned to control values with restoration of the afterload.

Glucose uptake. Figure 2 shows rates of glucose uptake (µmol·min⁻¹·g dry wt⁻¹) during four perfusion periods (from 0 to 15, 15 to 30, 30 to 60, and 60 to 90 min) with glucose as the only substrate (see Table 1). Because the experimental conditions for groups G₁₀, G₁₀-I-30, and G₁₀-I-60 were identical for the first 30 and 60 min of the protocol, values are reported collectively (n = 15 and n = 9, respectively). In hearts perfused with glucose (10 mM) alone (G₁₀), glucose uptake remained unchanged for the entire duration of the experiment, i.e., before, during, and after ischemia. The lack of stimulation of glucose uptake is probably due to a high baseline uptake when glucose is provided as the only substrate. Rates of glucose uptake with glucose alone are twice as high as the rates with glucose plus a cosubstrate (see Figs. 3 and 4).

The high baseline rate of glucose uptake in the glucose-alone group is explained largely by the absence of a competing substrate. In addition, the high concentration of glucose (10 mM) also contributed to the high baseline rate. Because the high baseline rate provides an explanation for the lack of stimulation by ischemia, we investigated whether glucose uptake could be stimulated by ischemia at a physiological concentration of glucose (5 mM). In the G₅ group, the preischemic rate of glucose uptake was 4.1 ± 1.0 µmol·min⁻¹·g dry wt⁻¹ (n = 9) and was not changed by ischemia (3.7 ± 0.8 µmol·min⁻¹·g dry wt⁻¹ from 15 to 30 min of the protocol, n = 4). Therefore, lack of stimulation by ischemia did not result from saturation of glucose utilization at twice the physiological concentration of glucose. The degree of insulin stimulation during ischemia at 5 mM glucose was, in absolute terms, similar to the stimulation at 10 mM glucose [the rate was increased from 3.7 ± 0.8 (n = 9) to 6.5 ± 1.2 µmol·min⁻¹·g dry wt⁻¹ (n = 4); P < 0.05], although based on a percent increase, the stimulation at 5 mM (71%) was more pronounced because the baseline rate was lower than at 10 mM glucose. At the higher glucose concentration, insulin stimulated glucose uptake before, during, and after ischemia by 58% (P < 0.01), 38% (P < 0.05), and 92% (P < 0.001), respectively (Fig. 2). The increase in glucose uptake was most pronounced when insulin was added at the beginning of reperfusion (G₁₀-I-60).

We also measured the release of lactate and alanine in the four groups. Lactate release was increased with low-flow ischemia (12.3 ± 3.8 vs. 5.9 ± 1.1 µmol·min⁻¹·g dry wt⁻¹; P < 0.001). However, stimulation of glucose uptake by insulin did not result in a further increase of lactate production [14.9 ± 3.1 vs. 12.3 ± 3.8 µmol·min⁻¹·g dry wt⁻¹; not significant (NS)]. Lactate production did not increase in nonischemic hearts with the addition of insulin (G₁₀-I-0; 6.3 ± 1.1 vs. 5.9 ± 1.1 µmol·min⁻¹·g dry wt⁻¹; NS). In the postischemic hearts,
lactate release returned to control values. Rates of alanine release were <0.01 μmol·min⁻¹·g dry wt⁻¹ before reperfusion in all groups. Alanine increased to detectable levels during reperfusion (up to 0.052 μmol·min⁻¹·g dry wt⁻¹), but rates remained minor compared with rates of lactate production.

Figure 3 depicts rates of glucose uptake in hearts perfused with glucose (10 mM) and lactate (5 min) alone (L) and with glucose and lactate plus insulin added at 30 min (L-I-30) or 60 min (L-I-60). In the presence of lactate, the initial rates of glucose uptake were one-half those observed for hearts utilizing glucose as the only substrate (compare with the first column of Fig. 2). In contrast to hearts perfused with glucose alone, with lactate as a cosubstrate glucose uptake nearly doubled during low-flow ischemia (4.9 ± 1.5 vs. 2.7 ± 1.2 μmol·min⁻¹·g dry wt⁻¹; P < 0.01).

Figure 4 depicts rates of glucose uptake in the groups perfused with glucose (10 mM) plus β-hydroxybutyrate (10 mM) (B groups) and with glucose plus octanoate (5 mM) (O groups), with and without insulin. The results in the presence of BOH or octanoate as a second substrate are qualitatively the same as with lactate, although lactate was somewhat less effective than the other compounds tested at inhibiting glucose uptake. Similar to lactate, the initial rate of glucose uptake was depressed on addition of BOH or octanoate (~73% and ~70%, respectively). In contrast to hearts perfused with glucose alone and similar to hearts perfused with glucose plus lactate, hearts subjected to low-flow ischemia had increased glucose uptake in the presence of BOH (127%; P < 0.05) and a trend toward increased uptake in the presence of octanoate (65%; NS). Thus it appears that glucose uptake is increased with ischemia only when there is a second substrate, similar to the situation in vivo.

Lactate release. Figure 5 shows rates of lactate release in hearts perfused with glucose (10 mM) alone, with glucose plus lactate, and with glucose and BOH. In hearts perfused with glucose alone, there was lactate release from the beginning of the perfusion. Lactate release increased with ischemia and decreased with reperfusion (Fig. 5A). The addition of insulin resulted in increased lactate release with reperfusion (8.8 ± 2.6 vs. 1.0 ± 1.2 μmol·min⁻¹·g dry wt⁻¹, P < 0.01). There was no net lactate uptake in hearts perfused with glucose and lactate during the control period (Fig. 5B). This is not unexpected because, in the well-oxygenated working heart, lactate competes effectively with glucose as the fuel for respiration. However, there was no net lactate uptake or release during ischemia. Therefore, glycolysis continued in the presence of added lactate. Reperfusion restored lactate uptake and oxidation. With BOH (Fig. 5C), there was no change in net lactate release with ischemia, but, during early reperfusion, lactate release changed to lactate uptake. Because ischemia stimulates glucose uptake in the presence of BOH (Fig. 4) without a corresponding increase in lactate release (Fig. 5), this suggests that BOH inhibits glycolysis more than glucose uptake. Note also that insulin had no effect on lactate release, except in hearts perfused with glucose as the only substrate, in which insulin increased the rate of lactate release.
Tissue metabolites. Because insulin had no effect on lactate release and because contractile performance (and presumably oxygen consumption) was the same in all groups, we were interested to know whether the increased glucose uptake after insulin treatment resulted in an increase in the glycogen content of the heart. In addition, we were interested in whether there were concomitant changes in levels of citrate, a regulator of glycolysis and, indirectly, of glycogen synthesis. The results are shown in Table 2.

Glycogen content increased significantly in hearts perfused with glucose and lactate plus insulin added after ischemia (L-I-60) as well as in hearts perfused with glucose and BOH plus insulin added during ischemia (B-I-30). There was no correlation among glucose-6-phosphate content, rates of glucose uptake, and tissue glycogen content (data not presented).

Citrate levels were significantly increased in hearts perfused with glucose and BOH either without (B) or with insulin (B-I-30). There was a trend toward increased citrate levels in hearts perfused with glucose and lactate, but the differences were not significant. This observation is in keeping with the greater inhibition of glucose uptake with BOH compared with lactate as cosubstrates (see Figs. 3 and 4).

DISCUSSION

We examined the uptake of glucose by the isolated, working heart in a model of reversible reduction in coronary flow. We found that coronary flow reduction increases glycolytic flux in the presence of cosubstrates, that insulin stimulates glucose uptake in the ischemic heart, and that insulin plus a cosubstrate of glucose promotes net glycogen synthesis in the postischemic heart. Although we assessed glycolytic flux only indirectly, the present results are consistent with the hypothesis that glucose is redirected from predominant oxidation to predominant lactate production although net glucose uptake remains unchanged (2).

Enhanced glucose uptake during low-flow ischemia with either lactate or BOH added and the change from lactate consumption to lactate production suggest that low-flow ischemia overcomes the restraint placed on glycolysis by competing substrates, predominantly at the level of phosphofructokinase (20). Allosteric control of phosphofructokinase is extensive and provides for large changes in catalytic activity. Citrate and ATP inhibit (18), whereas ADP, AMP, and, especially, fructose 2,6-bisphosphate activate, the enzyme (3). Although anoxia does not increase fructose 2,6-bisphosphate (3), the lack of O2 lowers intracellular pH, raises ADP and AMP, and makes the enzyme more sensitive to allosteric regulation (18). It is tempting to speculate

Table 2. Tissue metabolites

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>G10</th>
<th>G10-I-0</th>
<th>G10-I-30</th>
<th>G10-I-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen, µmol/g dry wt</td>
<td>106 ± 20</td>
<td>162 ± 39*</td>
<td>125 ± 13</td>
<td>124 ± 18</td>
</tr>
<tr>
<td>Citrate, nmol/g dry wt</td>
<td>759 ± 158</td>
<td>636 ± 188</td>
<td>810 ± 104</td>
<td>632 ± 161</td>
</tr>
<tr>
<td>Glycogen, µmol/g dry wt</td>
<td>125 ± 26</td>
<td>162 ± 44</td>
<td>181 ± 41*</td>
<td></td>
</tr>
<tr>
<td>Citrate, nmol/g dry wt</td>
<td>1,324 ± 354</td>
<td>1,954 ± 1,015</td>
<td>967 ± 327</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD of 4–6 experiments. Hearts were freeze-clamped at end of each perfusion protocol. See Table 1 for definitions of groups. Glycogen is expressed as micromoles of glycosyl units per gram of dry weight. *P < 0.05, †P < 0.01, and ‡P < 0.001 compared with G10 (glucose only).
that the rise in ADP and AMP observed with ischemia (26) may activate phosphofructokinase and override the inhibition by citrate.

The literature reports considerable differences between the effects of low-flow ischemia and no-flow ischemia on the activity of pyruvate dehydrogenase in the postischemic heart. The pyruvate dehydrogenase complex remains activated during no-flow ischemia (7) and becomes inactivated with reperfusion (10). In contrast, the complex becomes inactivated very early during low-flow ischemia, most likely as a result of activation of pyruvate dehydrogenase kinase (29). Our data using glucose and lactate as competing substrates show reversible lactate release during ischemia and suggest reversible inactivation of the pyruvate dehydrogenase complex with low-flow ischemia. The inhibition of glyceraldehyde-3-phosphate dehydrogenase by lactate which occurs during ischemia appears to be less pronounced than the inhibition of either phosphofructokinase or the pyruvate dehydrogenase complex, because lactate uptake changed to lactate release with ischemia.

Effects of insulin. The action of insulin leading to the substitution of glucose for fatty acids as the main fuel for respiration in heart muscle is well documented (1, 13, 14, 19, 20, 32), but the role of insulin in glucose uptake and metabolism by ischemic heart muscle is less certain.

We argued that if glucose extraction is increased with ischemia, insulin responsiveness may be blunted. We used net glucose uptake as a measure of insulin responsiveness because we found that in our model of low-flow ischemia (~70% reduction of coronary flow), there was no change in glucose uptake. Instead, glucose uptake was increased with ischemia only when a second substrate was present. This was an unexpected finding suggesting that low-flow ischemia (and presumably the resultant hypoxia) partially relieves the restraint imposed on glycolysis by competing substrates. Also, unexpectedly, we found that both the ischemic and the postischemic myocardium remained insulin responsive and that insulin promoted net glycogen synthesis in the postischemic heart. The reason for the discrepancy between our present data and those of Neely et al. (15) may be due to the fact that we used hearts from fasted animals or may be due to the low afterload of the heart in our model. Fasting in vivo improves ischemia tolerance of the heart in vitro. Our findings are consistent with the recent report (11) that glucose uptake can be increased strikingly by insulin in chronically dysfunctional, but viable, myocardium in patients with coronary artery disease.

These observations have both clinical and physiological implications. First, our model is a model of perfusion/metabolism mismatch. Although the model shows all the limitations of an in vitro model of myocardial ischemia, it exhibits the same characteristics as the pattern of blood "flow/metabolism mismatch" observed in vivo (11, 27). We note that the same principle of flow/metabolism mismatch also holds true when in vitro glucose uptake is increased relative to flow, i.e., when insulin was added at the beginning of reperfusion (Figs. 1 and 3). In this case, flow remained constant whereas glucose uptake doubled. The net result is a decrease in the ratio of flow to glucose uptake.

Second, the insulin-stimulated glucose uptake by the postischemic heart suggests that, during acute ischemia and reperfusion, muscle retains its insulin sensitivity. This observation is in agreement with the clinical observation of enhanced glucose uptake by the postischemic heart treated with glucose, insulin, and potassium (GIK). It is also in agreement with the increase in glucose uptake observed in a model of myocardial hibernation of the swine heart in which coronary flow was normal at rest but glucose uptake was increased by a factor of three (9). Although these authors did not measure plasma insulin levels, the relatively low levels of fatty acids (~0.4 µmol/ml) and the requirement for glucose supplementation suggest that pancreatic insulin production was increased in their animals.

Third, the mechanism for the enhanced glucose uptake during low-flow ischemia with glucose and a second substrate cannot be deduced from the present data. Although translocation of GLUT-4 to the plasma membrane has been demonstrated with total, global ischemia (24), it has only recently been shown (34) that this translocation is also associated with an increase in glucose uptake and glycolytic flux. Comparing the rates of glucose uptake in Figs. 2 and 3, as well as Fig. 4, brings up an interesting question. When glucose is the only substrate (G-1-0, G-1-30, and G-1-60 groups), rates of glucose uptake are greater than when cosubstrates are present. These data suggest that insulin produces a maximal degree of glucose transporter translocation to the plasma membrane, as has been observed in dog heart (34), whereas the effect of ischemia on translocation is less than maximal. The observation is consistent with the existence of two separate intracellular signaling pathways, one of which is activated by insulin and the other by the metabolic requirements of the heart. This indirect evidence is supported by observations (33) in skeletal muscle in which separate signaling pathways for insulin- and contraction-activated hexose transport have been described. In skeletal muscle, the contraction-activated pathway of glucose transport shares the same properties as the hypoxia-activated pathway.

Lactate metabolism. The data on lactate metabolism (Fig. 5) are of particular interest. As expected, lactate release was significantly increased during ischemia both with and without insulin. Unexpectedly, lactate release was observed only in the presence of insulin on reperfusion. This increase in lactate release is paralleled by an increase in glucose uptake and net glycogen content (assuming that glucose oxidation remains the same). When lactate was present as a cosubstrate with glucose (Fig. 5B), there was net lactate uptake under control conditions. During ischemia, lactate uptake changed to lactate release when insulin was added. This suggests continued glycolysis in the face of high extracellular lactate concentrations. Thus exogenous...
lactate could not have caused the inhibition of glycolysis described in the total, global ischemic heart (21). It is of interest that, during reperfusion, lactate uptake was less when insulin was present than when no insulin was present. BOH as a cosubstrate prevented was less when insulin was present than when no insulin was present. In this group, the most dramatic changes occurred during reperfusion. There was no lactate release in the absence of insulin (P < 0.001 compared with control). The results suggest that glucose was either oxidized or, more likely, incorporated into glycogen.

Physiological implications. Although we did not observe an inotropic effect of insulin, our findings have a number of physiological and clinical implications. Several studies in vivo support our findings indirectly, although none of them has directly examined the effects of insulin on the postischemic, reperfused myocardium. Best known among the studies are those of Sodi-Pallares (23) on the effects of intravenous infusions of GIK on the resolution of electrocardiographic signs of acute myocardial infarction. Glucose and insulin concentrations may act beneficially for the ischemic myocardium by increasing the rate of anaerobic glycolysis, reversing ion losses through a direct membrane effect, altering extracellular volume, and decreasing the circulating free fatty acid concentrations (7). Several workers have shown a protective effect of glycolysis during ischemia, resulting in an improved recovery of function on reperfusion of isolated, perfused rabbit hearts (4, 22, 28). Although there are no studies of insulin effects on the reperfused heart in vivo, the enhanced insulin responsiveness in the postischemic heart supports the clinical observation of enhanced glucose uptake and improved contractile function in reperfused heart muscle after aortocoronary bypass surgery performed with cardiopulmonary bypass (5).

Our observations also suggest that the stimulation of glucose uptake by insulin results in a net increase in glycogen. Both lactate and BOH promote glycogen resynthesis in the postischemic heart beyond the rate of glycogen resynthesis that we observed previously in hearts reperfused with glucose as the only substrate (2). Recent studies (12) on glucose metabolism in a canine model of low-flow myocardial ischemia have revealed stable rates of glucose oxidation but increased rates of lactate release and glycogen synthesis. Although the workers did not measure glucose uptake, our in vitro findings seem to be in good agreement with these data. Whereas the insulin stimulation of heart glycogen synthase D phosphatase is well known (16), it has also been observed that hypoxia activates heart glycogen synthase and glycogen synthesis (8) and that this effect probably accounts for the rapid posthypoxic glycogen synthesis. The physiological role of glycogen in cardiac function and metabolism is still largely unknown.

In conclusion, to our knowledge this is the first description of an insulin effect in heart muscle during ischemia and reperfusion associated with an increase in glycolytic flux and elevated glycogen content at the end of reperfusion. The study also shows that low-flow ischemia enhances glucose uptake and glycolysis, but only in the presence of a second substrate.

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