Protection of ischemic hearts by high glucose is mediated, in part, by GLUT-4

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REPERFUSION REMAINS the most effective therapy for treatment of evolving myocardial infarction. Nonetheless, the efficacy of reperfusion in limiting infarction and optimizing recovery of contractile function depends on the amount of irreversible damage occurring before initiation of reperfusion. Numerous studies have concluded that the irreversible damage occurring during ischemia is related to failure of energy production to meet even the basal needs of the jeopardized myocardium.

Our laboratory previously demonstrated (19, 27, 28) that diverse metabolic interventions that enhance glycolytic flux during ischemia diminish ischemic injury and enhance the salutary effects of reperfusion. Among numerous beneficial interventions, we observed that the use of high glucose in the perfusion medium was potent in attenuating ischemic injury and improving contractile function on reperfusion (27). During ischemia, the ability of the myocardium to generate sufficient high-energy phosphates through aerobic oxidation of fatty acids and carbohydrates is severely compromised (8, 13, 15, 27). Anaerobic metabolism of exogenous glucose then becomes an important method of generating ATP (8, 13, 15, 27). Recent clinical studies support the use of metabolic adjuncts to reperfusion therapy. In these studies (3), the administration of glucose-insulin-potassium significantly reduced mortality in patients with acute myocardial infarction (3), supporting the concept that metabolic interventions may be an important adjunctive therapy for protecting ischemic myocardium.

Myocardial glucose metabolism is dependent on the uptake of extracellular glucose, which is regulated by the transmembrane glucose gradient and the activity of glucose transporters GLUT-1 and GLUT-4 (8, 25, 26, 29). In the heart, GLUT-1 is relatively insulin insensitive and is considered to be responsible for basal glucose uptake in the setting of low fasting insulin concentrations and normal conditions (2, 14, 26, 29). GLUT-4, which is insulin sensitive, is distributed to a greater extent in the intracellular vesicles under normoxic conditions (2, 14, 26, 29). On stimulation by insulin or ischemia, GLUT-4 is translocated to the sarcolemma where it mediates increased glucose uptake into the myocyte (2, 14, 26, 29). In this study, we evaluated whether the protection of ischemic myocardium by perfusion with high glucose is associated with increased distribution of glucose transporters in the sarcolemma where it mediates increased glucose uptake.

Insulin sensitive glucose transporters; metabolism

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sarclemma, specifically GLUT-4. The results suggest that increased sarclemmal content of GLUT-4 is a key component of cardioprotection observed in hearts perfused with high glucose.

MATERIALS AND METHODS

All studies were performed with the approval of the Animal Care Committee at Columbia University. This investigation conforms with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 85-23, Revised 1996.

All chemicals were of the highest purity, were obtained from commercial sources, and were used without further purification except bovine serum albumin (BSA). Fatty acid-free BSA (from Amersham) was dialyzed for 48 h to decrease low-molecular-weight impurities. Furthermore, BSA preparations were tested to rule out any endotoxin contamination. Total long-chain fatty acid content of these BSA solutions were measured by gas chromatography and determined to be <0.02 mM. Final concentrations of long-chain fatty acids in the perfusion buffer was adjusted to 0.4 mM by adding palmitate.

Isolated heart preparation. Experiments were performed using an isovolumic isolated heart preparation as published earlier (6, 27, 28) and modified for use in rat hearts. Wistar rats (250–300 g) were anesthetized using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg). After deep anesthesia was achieved, hearts were rapidly excised, placed into iced saline, and retrogradely perfused in a nonrecirculating mode through the aorta at a rate of 12.5 ml/min. Constant flow perfusion was employed to rule out potentially confounding effects of variations in coronary flow. Hearts were perfused with nonrecirculating modified Krebs-Henseleit buffer containing 0.4 mM palmitate bound to equimolar albumin (US Biochemical; Cleveland, OH), 5 mM glucose, and 70 mM insulin (Eli Lilly; Indianapolis, IN).

The perfusate contained the following ions (in meq/l): 142 sodium, 123 chloride, 6 potassium, 2.5 calcium, 2 magnesium, 1.2 sulfate, 25 bicarbonate, and 1.4 phosphate. The perfusate was equilibrated with a gas mixture of 95% O2-5% CO2 to obtain a PO2 >600 mmHg. The buffer was maintained at 37°C throughout the experiments. Hearts were paced via the right atrium at 300 beats/min.

Left ventricular pressure was measured via a latex balloon inserted into the left ventricular cavity through the left atrium and attached to a pressure transducer (Gould Laboratories; Pasadena, CA). Coronary perfusion pressure was measured via a pressure transducer attached to the perfusate inflow line. A strip-chart physiograph recorder was used to record and measure heart rate, perfusion pressure, and left ventricular pressure.

Experimental protocol. After initial isolation and surgical procedures, hearts were perfused at a constant flow (12.5 ml/min) and were allowed to equilibrate for 30 min. Left ventricular end-diastolic pressure (LVEDP) was set at 4–8 mmHg by filling the left ventricular balloon with water. Ischemia was induced by perfusing hearts for 50 min at 5% of baseline flow (0.7 ml/min) with buffer equilibrated to room air and 5% CO2. This procedure was performed to achieve severe hypoxia during low-flow ischemia. After the ischemic period, hearts were reperfused for 60 min at the control flow rates (12.5 ml/min) and oxygenation conditions.

Four groups of hearts were studied. All hearts were perfused with equimolar amounts of palmitate and albumin as described above. The first group of hearts (controls) was perfused with control perfusate containing 5 mM glucose and 70 mM of insulin throughout the study. The second group of hearts (high glucose) were perfused with buffer containing 10 mM glucose and 70 mM of insulin. To determine the importance of insulin-regulable glucose transporters in mediating cardioprotection in hearts perfused with high glucose, the preferential inhibitor of insulin-regulable glucose transporters, cytochalasin B, was administered to the hearts with control or high-glucose perfusate starting 10 min before ischemia. Cytochalasin B, a fungal metabolite and well-recognized inhibitor of insulin-regulable transport of glucose (GLUT-4->GLUT-1) (2, 12, 30), was used at 10 μM concentration based on earlier dosing studies in our laboratory. In these studies, cytochalasin B at 10 μM did not influence cardiac systolic or diastolic function (data not shown). Furthermore, an earlier in vitro study (30) established that cytochalasin B has an inhibitory constant of ~200 nM for cardiac GLUT-4. Studies (2, 12, 30) in the literature have reported the use of 1–50 μM cytochalasin B in heart and muscle preparations. It was also demonstrated that the uptake and binding of cytochalasin-B is greater in tissue when the concentration of glucose in the sarcolemma is increased (30). Because it has been shown that ischemia-hypoxia increases sarclemmal content of glucose transporters (2, 14, 26, 30), we chose the dose such that under ischemic conditions cytochalasin B would be saturating and, thereby, achieve maximal inhibition of glucose transport.

Collection and analysis of perfusate and pulmonary effluent samples. Coronary venous effluent was collected via a cannula placed into the pulmonary artery. PO2, PCO2, and pH were measured in the effluent using an Instrumentation Laboratories IL-1306 pH-blood gas analyzer, whereas lactate production was measured using enzymatic methods (27). Myocardial oxygen consumption was calculated as (0.003 × arterial PO2 – 0.003 × effluent PO2) × total flow/left ventricular weight. The value 0.003 represents milliliters of O2 dissolved per deciliter of buffer. Lactate production, expressed as micromoles per gram wet weight per minute, was calculated from the perfusate-effluent differences, multiplied by flow, and divided by the left ventricular weight (10, 27). Creatine kinase release during reperfusion was measured using spectrophotometric assay as published earlier (27, 28).

Measurement of 2-deoxy[1-14C]glucose uptake. To determine the effect of cytochalasin B on myocardial glucose uptake, hearts were perfused with 2-deoxy[1-14C]glucose (nonradioactive glucose was 5 mM) in the presence and absence of insulin, and its uptake was determined. Briefly, the 2-deoxy[1-14C]glucose content was determined in the arterial perfusate before initiating recirculation. After the hearts were perfused for 60 min with 2-deoxy[1-14C]glucose in the recirculating mode, the perfusate was analyzed for 2-deoxy[1-14C]glucose. Scintillation cocktail (5 ml) (Ecoscint, National Diagnostics) was added to the samples and counted for 14C activity. The difference between the radioactive counts in the initial and final samples yield the amount of 2-deoxy[1-14C]glucose uptake in the heart.

Uptake of [3H]cytochalasin B. To determine whether perfusion with high glucose increases insulin-regulable glucose transporter activity, [3H]cytochalasin B uptake was measured in the following additional groups of hearts. In the first set of experiments, control hearts (n = 4) and high-glucose hearts (n = 4) were perfused with [3H]cytochalasin B (10 μCi in 100 ml) for 40 min in the recirculating mode under normoxic conditions. In the second set of experiments, control and high-glucose hearts were perfused in the recirculating mode with [3H]cytochalasin B (10 μCi in 100 ml) and subjected to hypoxia (buffer equilibrated with 95% air-5% CO2, PO2 = 185 mmHg) for 40 min.
GLUT-4 AND CARDIOPROTECTION

$[^{3}H]$cytochalasin B uptake was determined by analyzing the perfusate before initiating recirculation. After the hearts were perfused for 40 min with $[^{3}H]$cytochalasin B in the recirculating mode (100 ml total volume), heart tissue was analyzed for $[^{3}H]$cytochalasin B uptake. Frozen heart tissue was solubilized with NCS solubilizer (Amersham) (0.5 ml for every 50 mg of tissue) in a water bath at 55°C for 24 h. After cooling, 50 μl of glacial acetic acid were added, followed by 5-ml addition of scintillation cocktail. Radioactive counts were measured using a scintillation counter.

Subcellular membrane preparation. To evaluate the subcellular localization of GLUT-4 and GLUT-1, membrane fractions were prepared. Briefly, homogenates from the myocardium were prepared by homogenizing the tissue in a buffer containing sucrose (250 mM), sodium bicarbonate (10 mM), and sodium azide (5 mM), using an Ultra-Turrax Polytron homogenizer and centrifuging at 1,200 × g for 10 min. Pellets were rehomogenized and centrifuged again for 10 min. The supernatant, which constitutes the membrane fraction, was centrifuged at 190,000 × g for 1 h. The membrane pellets were resuspended in a 25% sucrose solution and loaded onto a discontinuous sucrose gradient (25%, 30%, and 35% wt/vol) and centrifuged for 20 h at 150,000 g. All procedures were performed at 4°C. The sarcolemma fraction was collected from the upper half of the 25% sucrose layer and the intracellular membranes from the 30% and 35% sucrose layers. The membrane fractions were harvested and diluted fivefold in sodium bicarbonate (10 mM)-sodium azide (5 mM) solution and centrifuged at 190,000 g for 1 h. The resulting membrane pellets were resuspended in a sucrose (250 mM)-Tris buffer (50 mM, pH 7.4).

Marker enzyme assays. Total protein content was determined using the Bradford protein assay kit (Pharmacia). The activities of 5'-nucleotidase and NADPH-cytochrome c reductase were measured as respective markers of sarcolemmal and intracellular membrane preparations. The activities of these markers were assayed according to published protocols (30).

GLUT-4 and GLUT-1 immunoblot analysis. To assess changes in GLUT-4 and GLUT-1 protein expression in control and high-glucose-perfused hearts, SDS-PAGE gel analysis was performed on sarcolemma and intracellular membranes (20 μg protein) with 10% gels under reducing conditions. The protein samples were diluted with buffer containing 2% SDS with 3% thiothreitol to prevent protein aggregation. The gels were run at 200 V for 45 min in a minigel electrophoresis apparatus. Proteins were transferred to polyvinylidene difluoride membranes (Trans-blot membranes, Bio-Rad) at 200 mA for 1 h. Membranes were blocked initially for 1 h with 5% milk in PBS buffer at 37°C and then with 1% milk in PBS buffer overnight at 4°C. Membranes were washed with (in mM) 136 NaCl, 2.7 KCl, 1.5 KH2PO4, 8 Na2HPO4, and 3 NaN3, and 1% Triton X-100, and then incubated with primary antibodies of GLUT-4 or GLUT-1 (Biogenex). The membranes were washed with and incubated with 2 μCi of $^{32}$P-labeled protein-A with 1% milk in PBS at 25°C for 1 h. They were then washed, air-dried, and autoradiographed with XAR-5 film (Eastman Kodak) for 12 h at −80°C with double-intensifying screens. The bands were excised from the polyvinylidene difluoride membrane and counted in a gamma counter (Beckman). The counts were corrected for background activity.

Northern blots for GLUT-4 mRNA expression. To measure changes in GLUT-4 expression, RNA was obtained from the heart homogenates as described in the commercial RNA kit. Briefly, 20 μg of total RNA from each sample were electrophoresed on 6% formaldehyde, 1% agarose gels, and then transferred with 10× SSC (150 mmol/l NaCl, 15 mmol/l sodium citrate, pH 7.0) to nylon membrane. The blots were hybridized with full length, uniformly $^{32}$P-labeled rat GLUT-4 antisense cRNA probes in 50% formamide hybridization solution at 65°C overnight. The blots were washed four times for 15 min in 0.1× SSC, 0.1% SDS at 65°C. These conditions were employed to obtain specific detection of GLUT-4 mRNA without cross-hybridization.

Statistical analysis. Values are expressed as means ± SD. Significance of differences were determined using one-way analysis of variance for repeated measurements with additional post hoc tests for differences. $P$ values <0.05 were considered statistically significant.

RESULTS

Hemodynamics. LVDP and LVEDP were similar in all groups under baseline conditions (Table 1). Reduction of perfusate flow resulted in cessation of LVDP in all hearts. As previously demonstrated by us (20, 27, 28), perfusion of hearts with high glucose resulted in attenuation of the rise in LVEDP during ischemia and improved LVDP recovery on reperfusion ($P = 0.04$). Inhibition of insulin-regulable glucose transporters with cytochalasin B abolished recovery of LVDP in high-glucose-perfused hearts.

Myocardial oxygen consumption was similar in all groups under baseline and ischemic conditions and was unaffected by perfusion with high glucose or with cytochalasin B (Table 1).

Creatine kinase release. Creatine kinase release during reperfusion was used as a measure of ischemic injury in our studies. Similar to our earlier findings (27, 28), the data presented here demonstrated reduced ischemic injury in hearts perfused with high glucose (Fig. 1). In hearts perfused with cytochalasin B, the ischemic protection afforded by treatment with high glucose was abolished (Fig. 1).

Lactate release during ischemia. We previously demonstrated that continued lactate release during ischemia is a necessary prerequisite for maintained metabolic viability, decreased contracture, and enhanced function on reperfusion (20, 27, 28). Treatment with high glucose was associated with enhanced lactate release during ischemia (Fig. 2), reflecting maintained anaerobic metabolism. Cytochalasin B prevented ongoing lactate release during ischemia in high-glucose-perfused hearts (Fig. 2).

Effect of cytochalasin B on baseline myocardial glucose uptake. To determine whether cytochalasin B inhibits insulin-independent glucose uptake, control and cytochalasin B treated hearts were perfused with 2-deoxy[1−14C]glucose in the presence and absence of insulin for 60 min. Table 2 shows that the presence of insulin increased 2-deoxy[1−14C]glucose uptake by ~3.5-fold. In the presence of insulin, cytochalasin B reduced 2-deoxy[1−14C]glucose uptake by ~3.4-fold. In the absence of insulin, the reduction in 2-deoxy[1−14C]glucose uptake by cytochalasin B was also 3.5-fold. These data suggest that cytochalasin B does not completely abolish glucose uptake in isolated perfused hearts.
Assessment of glucose transporters by radioactive cytochalasin B uptake. To further define the involvement of glucose transporters, hearts were perfused with tracer amounts of \(^{3}H\)cytochalasin B and myocardial uptake of tracer was determined. \(^{3}H\)cytochalasin B uptake was increased in hearts perfused with high glucose not subjected to ischemia (Fig. 3). Hypoxia (decreased oxygen in the perfusate but with control levels of flow) also increased cytochalasin B uptake and the combination of high glucose and hypoxia was additive. The increased myocardial cytochalasin B uptake in high glucose and hypoxia hearts is consistent with the data published by Zaninetti et al. (30). In that study, it was demonstrated that the total binding of \(^{3}H\)cytochalasin B increases in cardiac tissue with stimulation of glucose transporters (30). Furthermore, it was also demonstrated that in insulin-stimulated cardiac tissue, the \(^{3}H\)cytochalasin B binding affinity with the data published by Zaninetti et al. (30). In that study, it was demonstrated that the total binding of \(^{3}H\)cytochalasin B increases in cardiac tissue with stimulation of glucose transporters (30).

Table 1. Hemodynamic values from isolated rat hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Measure</th>
<th>Equilibration</th>
<th>Ischemia, 50 min</th>
<th>Reperfusion, 60 min, (% equilibration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>LVDP</td>
<td>88 ± 12</td>
<td>15 ± 18</td>
<td>55 ± 12 (62 ± 19%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LVEDP</td>
<td>3 ± 2</td>
<td>82 ± 26</td>
<td>21 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MVO₂</td>
<td>6.2 ± 1.0</td>
<td>5.8 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PP</td>
<td>50 ± 6</td>
<td>68 ± 10 (80 ± 11%)</td>
<td></td>
</tr>
<tr>
<td>High glucose</td>
<td>6</td>
<td>LVDP</td>
<td>85 ± 10</td>
<td>2 ± 2*</td>
<td>1 ± 2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LVEDP</td>
<td>3 ± 2</td>
<td>6.8 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MVO₂</td>
<td>7.3 ± 1.8</td>
<td>5.2 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PP</td>
<td>54 ± 9</td>
<td>95 ± 14</td>
<td></td>
</tr>
<tr>
<td>Control + Cyt-B</td>
<td>5</td>
<td>LVDP</td>
<td>99 ± 21</td>
<td>43 ± 15*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LVEDP</td>
<td>2 ± 2</td>
<td>41 ± 15†</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MVO₂</td>
<td>5.9 ± 0.6</td>
<td>37 ± 14**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PP</td>
<td>55 ± 9</td>
<td>44 ± 15 (44 ± 16%)</td>
<td></td>
</tr>
<tr>
<td>High glucose + Cyt-B</td>
<td>6</td>
<td>LVDP</td>
<td>102 ± 9</td>
<td>41 ± 10†</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LVEDP</td>
<td>2 ± 2</td>
<td>6.8 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MVO₂</td>
<td>6.1 ± 0.4</td>
<td>37 ± 14**</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PP</td>
<td>50 ± 5</td>
<td>100 ± 19</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; n = number of rats. Cyt B, cytochalasin B; LVDP, left ventricular developed pressure (mmHg); LVEDP, left ventricular end-diastolic pressure (mmHg); MVO₂, myocardial oxygen consumption (\(\mu\)mol·g wet wt\(^{-1}\)·min\(^{-1}\)); PP, perfusion pressure (mmHg). *P < 0.03 compared with controls; †P < 0.01 compared with high glucose.

Assessment of glucose transporters by radioactive cytochalasin B uptake. To further define the involvement of glucose transporters, hearts were perfused with tracer amounts of \(^{3}H\)cytochalasin B and myocardial uptake of tracer was determined. \(^{3}H\)cytochalasin B uptake was increased in hearts perfused with high glucose not subjected to ischemia (Fig. 3). Hypoxia (decreased oxygen in the perfusate but with control levels of flow) also increased cytochalasin B uptake and the combination of high glucose and hypoxia was additive. The increased myocardial cytochalasin B uptake in high glucose and hypoxia hearts is consistent with the data published by Zaninetti et al. (30). In that study, it was demonstrated that the total binding of \(^{3}H\)cytochalasin B increases in cardiac tissue with stimulation of glucose transporters (30). Furthermore, it was also demonstrated that in insulin-stimulated cardiac tissue, the \(^{3}H\)cytochalasin B binding affinity with the data published by Zaninetti et al. (30). In that study, it was demonstrated that the total binding of \(^{3}H\)cytochalasin B increases in cardiac tissue with stimulation of glucose transporters (30).

Fig. 1. Release of creatine kinase during reperfusion in control (CON, \(n = 18\)), high-glucose-perfused (HG, \(n = 6\)), CON + cytochalasin B (CB, \(n = 5\)), and HG + CB (\(n = 6\)) hearts. HG-treated hearts had diminished creatine kinase release, indicative of the metabolic protection of this intervention. However, pretreatment of hearts with CB ameliorated this response, suggesting the central role of insulin-regulable transporters in mediating protection during ischemia. Data are reported as means ± SD. *P = 0.02 vs. all other groups.

Fig. 2. Plot of lactate production during ischemia in CON (\(n = 18\)), HG (\(n = 6\)), CB (\(n = 5\)), or HG + CB (\(n = 6\)) hearts. Continued lactate production during ischemia is beneficial because it represents continued anaerobic metabolism. Blockade of the insulin-regulable glucose transporters ameliorated the beneficial metabolic effect of HG treatment. Data are reported as means ± SD. *P = 0.04 vs. all other groups.
increases eightfold in plasma membrane fraction (30). Our observations suggest that perfusion with high glucose increases the translocation of glucose transporters to the sarcolemma under normoxic as well as hypoxic conditions, and that the combination synergistically increases transporter number and/or affinity for cytochalasin B.

**Characterization of sarcolemmal and intracellular membrane preparations.** Sarcolemmal membranes were highly enriched for plasma membrane 5'-nucleotidase activity compared with the crude membrane preparation, whereas intracellular membranes were enriched with NADPH-cytochrome c reductase (Table 3). Enzyme markers for sarcolemmal and intracellular membranes were comparable in control and high-glucose-perfused hearts (Table 3). The protein content for these preparations were comparable in both groups of hearts.

![Graph showing normalized 3H cytochalasin B uptake](image)

Fig. 3. Histogram of tritiated cytochalasin B in uptake in CON (n = 4) or hearts perfused with HG (n = 4), hypoxia (HYP, n = 4), or HG + HYP (n = 4). Uptake was increased in hearts subjected to hypoxia at control flows as well as in hearts subjected to high exogenous glucose. There was an additive response with both HYP and HG. These results suggest that regulable glucose transporters are recruited with these interventions. *P < 0.04 vs. CON hearts, #P = 0.04 vs. HG hearts, **P = 0.03 vs. HYP hearts. Data are reported as means ± SD.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>2-[1-14C]deoxyglucose Uptake, nmols min⁻¹ g dry wt⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>327 ± 96</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>92 ± 17</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>1,130 ± 302</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>359 ± 43</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = number of rats; Group A, control, without insulin; Group B, control without insulin + cytochalasin B; Group C, control with insulin; and Group D, control with insulin + cytochalasin B. *P < 0.01 group B compared with all other groups; †P < 0.02 compared with groups A and D.

- **Table 2. 2-[1-14C]deoxyglucose uptake in the presence of cytochalasin B**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>5'-Nucleotidase Specific Activity, μU/mg</th>
<th>NADPH-Cytochrome c Reductase, μU/mg</th>
<th>Total Protein, mg/g heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>38.8 ± 5.4</td>
<td>6.2 ± 0.7</td>
<td>172.6 ± 12.1</td>
</tr>
<tr>
<td>Sarcolemmal</td>
<td></td>
<td>1.8 ± 0.3</td>
<td>24.3 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Intracellular</td>
<td></td>
<td>0.9 ± 0.3</td>
<td>2.1 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>High glucose</td>
<td>8</td>
<td>32.9 ± 6.6</td>
<td>7.2 ± 1.4</td>
<td>180.4 ± 17.7</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD; n = number of rats.

- **Table 3. Characterization of sarcolemmal and intracellular membrane preparations**

**GLUT-4 and GLUT-1 expression studies.** To determine whether the increases in cytochalasin B uptake is due to increases in GLUT-4 levels, we measured GLUT-4 protein expression in flash-frozen control hearts and in hearts perfused with high exogenous glucose. The blots shown in Fig. 4 demonstrate changes in GLUT-4 protein expression in different membrane fractions, with the sarcolemmal fraction being the most important one because it represents translocation of the transporter to the myocyte surface. As shown in Fig. 4, perfusion with high glucose increases the amount of GLUT-4 in the sarcolemmal fraction under baseline conditions as well as with ischemia. These data are consistent with the results from radioactive cytochalasin B studies suggesting that increased uptake of labeled cytochalasin B reflects increased GLUT-4 protein expression and/or translocation. GLUT-4 increased with either high exogenous glucose or with ischemia, and the combination was additive. These data suggest that one mechanism by which high exogenous glucose may enhance glycolysis is by increasing the GLUT-4 transporter.

To determine whether the increases in GLUT-4 protein content in high glucose and ischemic hearts are due to increased GLUT-4 mRNA expression, we performed Northern blot studies. The GLUT-4 mRNA expression changes in control, high glucose, ischemic, and high-glucose + ischemic hearts are shown in Table 4. Ischemia increased the glucose transporter mRNA expression in control and high-glucose hearts. Perfusion with high glucose did not significantly increase the GLUT-4 mRNA expression under baseline conditions. These mRNA expression data along with increased GLUT-4 protein expression observed in immunoblot studies, suggest that increased GLUT-4 protein expression is likely due to increased translation of GLUT-4 and or decreased GLUT-4 protein degradation.

Because GLUT-1 also plays a role in mediating glucose uptake, we examined whether increased GLUT-1 levels in the sarcolemma may play a role in the cardioprotection observed in high-glucose-perfused hearts. GLUT-1 was observed in both sarcolemmal and intracellular membrane fractions (Fig. 5). Perfusion with high-glucose buffer did not affect sarcolemmal GLUT-1. Ischemia increased sarcolemmal GLUT-1.
content, a finding similar to that published in the literature (29). In hearts perfused with high glucose and subjected to ischemia, a decrease in sarcolemmal GLUT-1 content and an increase in intracellular membrane fraction GLUT-1 were observed. Because the mRNA content was not different among groups, the data suggest that high glucose in ischemic hearts reduced translocation of GLUT-1 into the sarcolemma.

**DISCUSSION**

Biochemical and structural alterations occur in ischemic myocardium during the transition from the viable to the necrotic state. In the progress toward cell death, the myocardium passes through a phase of reversible and then irreversible ischemic injury. Studies from our laboratory and from others have demonstrated that myocardial energy depletion heralds the onset of irreversible ischemic injury (5, 9, 15, 18, 19, 21, 25, 27, 28). We and others have demonstrated that interventions that promote glucose use and glycolysis

**Table 4. GLUT-4 mRNA levels in control and high-glucose hearts**

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 9</td>
<td>172 ± 27*</td>
</tr>
<tr>
<td>High glucose</td>
<td>112 ± 12</td>
<td>198 ± 39*</td>
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</tbody>
</table>

Data are reported as means ± SD; n = 5 rats. Values are expressed relative to the amount of GAPDH mRNA loaded on the gel. The ratio of GLUT-4 mRNA/GAPDH mRNA in control hearts under baseline conditions were assigned a value of 100. All other hearts were compared with this set of control hearts. *P < 0.03 vs. control hearts under baseline conditions.

Fig. 4. Immunoblots and bar graphs of GLUT-4 content in intracellular (A) and sarcolemmal (B) membrane fractions from hearts perfused under control conditions or with HG, ischemia (ISC), or HG + ISC. Five hearts from each group were used for these immunoblot studies. In the intracellular membrane fraction, *P = 0.02 compared with CON and ISC. In the sarcolemmal membrane fraction, *P = 0.04 compared with CON, #P = 0.04 compared with HG, and @P = 0.04 compared with ISC.

Fig. 5. Immunoblots and bar graphs of GLUT-1 content in intracellular (A) and sarcolemmal (B) membrane fractions from hearts perfused under control conditions or with HG, ISC, or HG + ISC. Five hearts from each group were used for these immunoblot studies. In the intracellular membrane fraction, *P < 0.04 compared with CON. In the sarcolemmal membrane fraction, *P = 0.03 compared with CON, HG, and HG + ISC.
maintain ATP, prevent the onset of ischemic contracture, and lessen ischemic damage. These events set the stage for more rapid and complete recovery of contractile function during reperfusion (5, 9, 15, 18, 19, 21, 25, 27, 28). One of the interventions proven to be cardioprotective was perfusion of hearts with high glucose (27). In this study, we demonstrate that increased exogenous glucose concentrations result in increased translocation of GLUT-4 in the sarcolemmal membrane.

**Glucose transporters and glycolysis in ischemic hearts.** Because the availability of oxygen during flow-regulated ischemia is reduced, the ability of the myocardium to produce energy from oxidation of fatty acids and carbohydrates is greatly reduced (8, 11, 13, 25). Accordingly, during ischemia most of the energy production results from glucose metabolism via anaerobic glycolysis. Although glycolytic flux increases at the onset of ischemia, the increase is short lived, and glycolytic flux decreases during the later stages of ischemia. In this study, lactate production increased initially in control hearts within 5 min after the onset of ischemia but decreased with 50 min of ischemia. In comparison, lactate production in hearts perfused with high glucose remained steadily high during ischemia, reflecting maintained glycolysis in these hearts. Because we employed low-flow ischemia in a nonrecirculating perfusion mode, it is more likely that the maintained glycolysis in high-glucose hearts is due to increased exogenous glucose use (20, 27, 28). The increases in lactate release in hearts perfused with high glucose was associated with increases in translocation of GLUT-4. Previous studies have demonstrated that GLUT-4 translocation in the plasma membrane and glucose uptake are increased by ischemia and high insulin (14, 23, 26, 29). In this study, we demonstrate that high levels of exogenous glucose increases GLUT-4 content in the sarcolemmal membrane and that the increases during ischemia are additive. In contrast, sarcolemmal GLUT-1 content was decreased in ischemic hearts perfused with high glucose. Also, inhibition of glucose transporters by cytochalasin B (with greater affinity for insulin-sensitive transporters) completely abrogated the beneficial effects of high glucose. Thus the results of these studies suggest the importance of changes in sarcolemmal insulin-sensitive glucose transporters as a key contributing factor of the cardioprotection afforded by high-glucose perfusion.

**Metabolic and clinical importance.** In this study, we demonstrate an important mechanism responsible for the protective effect of increased exogenous glucose during ischemia. The transsarcolemmal glucose gradient along with the number of glucose transporters present in the sarcolemma determine the extent of glucose uptake. Studies have shown that GLUT-4 translocation increases glucose extraction during ischemia despite decreased glucose delivery and lower interstitial glucose concentrations (2, 16, 28). The findings in this study indicate that high exogenous glucose as well as a combination of high-glucose perfusion and ischemia increases recruitment of GLUT-4 to the sarcolemma, thereby increasing the ability of hearts to utilize extracellular glucose (2, 20).

The beneficial effect of high-glucose perfusion on functional and metabolic recovery is consistent with previous reports demonstrating a benefit of maintaining or enhancing glycolysis (1, 16, 17, 19, 22, 24, 25, 27, 28). In these studies, it was demonstrated that increasing glycolytic flux during ischemia prevented ischemic contracture, increased and/or maintained high-energy phosphates, reduced ischemic injury, and enhanced functional recovery on reperfusion. The beneficial effects of increasing glycolytic flux by enhancing glucose availability have been exploited clinically with the use of glucose-insulin-potassium infusions in the setting of myocardial infarction and cardiac surgery (3, 24). The data presented here provide further support of the use of high-glucose infusion in patients with myocardial ischemia.

**Study limitations.** The findings in this study should be interpreted within the framework of the experimental protocol employed. The use of cytochalasin B to demonstrate the importance of GLUT-4 should be interpreted with caution because cytochalasin B can also inhibit GLUT-1 mediated uptake, albeit to a lesser degree. However, our immunoblot data, demonstrating increased sarcolemmal GLUT-4 and decreased GLUT-1, strongly suggests that the protection of ischemic hearts perfused with high glucose is associated with greater sarcolemmal GLUT-4 content.

Whereas the abrogation of the beneficial effect of high-glucose perfusion by cytochalasin B is largely due to inhibition of glucose uptake during ischemia, the other effects of cytochalasin B must also be taken into account. For example, the binding of cytochalasin B has been shown to influence cell permeability and ATP-sensitive K⁺ (KATP) channel activity in cell culture and in vitro tissue preparations. Based on the dose used in this study, it is unlikely that the effects of cytochalasin B on cell permeability or on KATP channels played a major role in reversing the beneficial effects of high-glucose perfusion.

Another limitation of our study is the absence of lactate in perfusion medium. Studies have shown that lactate can be a significant source of energy (7) and that lactate can influence glucose uptake in cardiomyocytes (4). The extent of GLUT-4 translocation during ischemia observed in our study is similar to that demonstrated by Young et al. (29) in an in vivo dog model of ischemia. However, further studies are needed to investigate whether exogenous lactate differentially influences GLUT-4 translocation during ischemia in high-glucose-perfused hearts.

The data presented here demonstrate that perfusion with high glucose causes translocation of GLUT-4, resulting in increased glycolysis and protection from ischemia. Furthermore, we show that perfusion with high glucose and ischemia have additive effects on GLUT-4 translocation. GLUT-1 translocation to the sarcolemma is decreased in high-glucose-perfused ischemic hearts. Thus our studies show that changes in
GLUT-4 are a key component and not the sole mediator of cardioprotection in high-glucose-perfused hearts. These findings help explain the adaptation of heart tissue to ischemia and suggest a potential role for agents that upregulate GLUT-4 as a novel metabolic adjunct in the treatment of myocardial ischemia.

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