High Levels of Fatty Acids Delay the Recovery of Intracellular pH and Cardiac Efficiency in Post-Ischemic Hearts by Inhibiting Glucose Oxidation

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OBJECTIVES This study was designed to determine if the fatty acid-induced increase in H⁺ production from glycolysis uncoupled from glucose oxidation delays the recovery of intracellular pH (pHi) during reperfusion of ischemic hearts.

BACKGROUND High rates of fatty acid oxidation inhibit glucose oxidation and impair the recovery of mechanical function and cardiac efficiency during reperfusion of ischemic hearts.

METHODS pHᵢ was measured by ³¹P nuclear magnetic resonance spectroscopy in isolated working rat hearts perfused in the absence (5.5 mmol/l glucose) or presence of 1.2 mmol/l palmitate (glucose+palmitate). Glycolysis and glucose oxidation were measured using [5-³H/U-¹⁴C]glucose.

RESULTS When glucose+palmitate hearts were subjected to 20 min of no-flow ischemia, recoveries of mechanical function and cardiac efficiency were significantly impaired compared with glucose hearts. Glucose oxidation rates were significantly lower in glucose+palmitate hearts during reperfusion compared with glucose-perfused hearts. Dichloroacetate (3 mmol/l) given at reperfusion to glucose+palmitate hearts resulted in a 3.2-fold increase in glucose oxidation, a 35% ± 3% decrease in pHi production from glucose metabolism, a 1.7-fold increase in cardiac efficiency and a 2.2-fold increase in the rate of pHi recovery during reperfusion.

CONCLUSIONS A high level of fatty acid delays the recovery of pHi during reperfusion of ischemic hearts because of an increased H⁺ production from glycolysis uncoupled from glucose oxidation. Improving the coupling of glucose metabolism by stimulating glucose oxidation accelerates the recovery of pHi and improves both mechanical function and cardiac efficiency. (J Am Coll Cardiol 2002;39:718–25) © 2002 by the American College of Cardiology

During ischemia, anaerobic glycolysis is an important source of adenosine triphosphate (ATP) production (1). However, H⁺ production from the hydrolysis of glycolytically produced ATP also contributes to the acidosis that occurs in ischemic myocardium (2). Development of cellular acidosis decreases cardiac pressure development (3) and provokes cardiac arrhythmias (4). Acidosis can also decrease contractility by decreasing the response of contractile proteins to Ca²⁺, predominantly by a decrease in Ca²⁺+ binding to troponin C as protons compete with Ca²⁺+ at Ca²⁺+-binding sites (4). Intracellular acidosis during severe ischemia also increases sarcoplasmic Na⁺/H⁺ exchange (5,6). If the myocardium is reperfused, extracellular pH quickly normalizes, creating a large pH gradient across the membrane, and the resultant activation of Na⁺/H⁺ exchange increases intracellular Na⁺. This Na⁺ then alters Na⁺/Ca²⁺ exchange activity, leading to intracellular Ca²⁺ overload and cell death (5,6). Inhibition of Na⁺/H⁺ exchanger improves the recovery of cardiac function and efficiency during reperfusion (7–9), emphasizing that the accumulation of intracellular H⁺ during ischemia is an important contributing factor to ischemic injury. Whether continued production of H⁺ during the critical early period of reperfusion also has the potential to exacerbate ischemia-reperfusion injury has not been established.

In most clinical situations of reperfusion after ischemia, the heart muscle is exposed to high levels of fatty acids (10). Reperfusion of reversibly injured ischemic muscle results in a rapid recovery of fatty acid oxidation, with rates often exceeding pre-ischemic levels (11,12). This high rate of fatty acid oxidation inhibits the rate of glucose oxidation to a much greater extent than the rate of glycolysis, resulting in a marked uncoupling between the rates of glycolysis and glucose oxidation (13–16). This uncoupling of glucose metabolism is a potentially important source of H⁺ production during reperfusion (13,15,16). If glycolysis is coupled to glucose oxidation, H⁺ production from hydrolysis of ATP derived from glucose metabolism is zero (13,17). However, if glycolysis is uncoupled from glucose metabolism (and pyruvate derived from glycolysis is not oxidized), there is a net production of two H⁺ from each glucose molecule metabolized. As a result, high rates of fatty acid oxidation during the actual reperfusion period have the potential to

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increase H⁺ production generated from uncoupled glucose metabolism (13–16). Whether this translates into differences in pHᵢ recovery after ischemia is not known.

Although ³¹P-nuclear magnetic resonance (³¹P-NMR) is the standard for pHᵢ measurements, to our knowledge no previous studies have used this technique to assess directly the effects of fatty acids on rates of pHᵢ recovery after ischemia. We therefore directly measured pHᵢ in isolated working rat hearts in the presence and absence of a high level of fatty acid. Using this approach we compared rates of pHᵢ recovery after ischemia to rates of H⁺ production calculated from measurements of glycolysis and glucose oxidation. We also determined whether stimulation of glucose oxidation with dichloroacetate (DCA), a pyruvate dehydrogenase activator (14,18), could improve the recovery of cardiac function and efficiency by accelerating the recovery of pHᵢ during reperfusion.

**METHODS**

**Isolated working rat hearts.** Rat hearts were cannulated for isolated working heart perfusions, as described previously (13). All procedures on animals conformed with the guidelines of the Canadian Council on Animal Care and were approved by the University of Alberta Health Sciences Animal Policy Committee. In brief, male Sprague-Dawley rats (0.3 to 0.35 kg) were anesthetized with pentobarbital sodium (60 mg/kg i.p.), and the hearts were quickly excised, cannulated as working hearts, and perfused at a 11.5 mm Hg left atrial pre-load and 80 mm Hg aortic after-load. Spontaneously beating working hearts were perfused with Krebs-Henseleit solution containing 2.5 mmol/l calcium, 5.5 mmol/l glucose and 3% bovine serum albumin, in the presence or absence of 1.2 mmol/l palmitate, as described previously (19). Heart rate, aortic pressure, cardiac output, aortic flow, coronary flow, coronary resistance, myocardial O₂ consumption (MVO₂), cardiac work and cardiac efficiency were determined, as described previously (14,19).

The isolated working hearts were adapted for use within the confines of a magnet by methods described in the literature (20,21). Because measurement of glucose metabolism using radiolabeled tracers requires that the whole perfusion system to be sealed (22), we developed a perfusion system that permitted efficient operation of a closed system within the confines of a high field magnet. Identical perfusion systems were used for the metabolic and ³¹P NMR studies. The detailed tubing connections for the working heart have been shown previously (20,21). To optimize the performance of the working heart within the magnet, the oxygenator was placed within the bore of the magnet 15 cm above the heart, thereby providing the appropriate pre-load. The bottom part of the cannula assembly (heart chamber) was designed to fit within a 25-mm NMR tube. Coronary flow entered the space around the heart and was removed by a suction tube working directly off a peristaltic pump. Thus, the heart was always surrounded by perfusate up to the level of the aorta. In addition, the aortic outflow line, after leaving the compliance chamber, proceeded out of the magnet and into the perfusate reservoir for recirculation. This aortic outflow line provided the resistance necessary to generate 80 mm Hg hydrostatic pressure (afterload) on the heart. Perfusion lines were water-jacked to maintain perfusate temperature of 37°C. Total perfusate volume in the recirculating system was 150 ml.

**Experimental protocol.** Working hearts were initially perfused for 30 min under aerobic conditions. Global no-flow ischemia was then introduced by clamping both the left atrial inflow and aortic outflow lines. The left atrial inflow was clamped by an extension rod attached to a clamp positioned between the oxygenator and the heart. After 20 min of no-flow ischemia (33°C), the left atrial and aortic flows were restored, and the hearts were reperfused for 40 min under aerobic conditions. Experimental groups included: 1) 5.5 mmol/l glucose throughout (glucose); 2) 5.5 mmol/l glucose and 1.2 mmol/l palmitate throughout (glucose+palmitate); and 3) 5.5 mmol/l glucose and 1.2 mmol/l palmitate throughout, with 3 mmol/l DCA (BDH Chemicals Ltd., Toronto, Canada) added immediately before post-ischemic reperfusion (glucose+palmitate+DCA).

At the end of reperfusion, hearts were quickly frozen with Wollenberger clamps cooled to the temperature of liquid N₂ for dry weight-to-wet weight ratio determinations.

**Measurement of glycolysis and glucose oxidation.** Glycolysis and glucose oxidation were measured simultaneously by perfusing hearts with [S⁻¹H/U⁻¹⁴C] glucose (13,22). The total myocardial H₂O production and CO₂ production were determined at 10-min intervals during both the initial aerobic perfusion period and the 40-min period of reperfusion.

If glucose passes through glycolysis to lactate and the ATP so formed is hydrolyzed, a net production of 2 H⁺ per molecule of glucose occurs (10,14). In contrast, if glycolysis is coupled to glucose oxidation, the net production of H⁺ is zero. Therefore, the overall rate of H⁺ production derived from glucose utilization was calculated by subtracting the rate of glucose oxidation from the rate of glycolysis and multiplying by 2.

**Determination of pHᵢ by ³¹P-NMR spectroscopy.** ³¹P-NMR spectra were acquired using a Bruker Advance 500 spectrometer in conjunction with a 120 mm vertical bore.
11.7T magnet (Magnex, Oxford, U.K.). Under the same perfusion conditions as used for the metabolic studies, the working hearts were positioned within a 25-mm dual channel NMR probe (Morris Instruments, Gloucester, Ontario, Canada). Cardiac function was comparable in both series of experiments. Field homogeneity was adjusted by shimming on the proton signal using the $^1$H channel and yielded line widths of approximately 0.1 ppm. $^{31}$P spectra were acquired at 202.4 MHz with a time resolution of 2.25 min (1.12 min at the end of ischemia and first 10 min of reperfusion), using a 60° pulse and a 1.8-s recycle time. Spectra were processed using WIN NMR (Bruker) by summing 72 (or 36) free induction decays and subjected to Fourier transform after exponential multiplication (line broadening = 30). The content of high energy phosphates was determined by integration of the areas under the peaks. During an initial stabilization period of 30 min aerobic perfusion, which included the time required for tuning of the probe and shimming of the magnet, baseline spectra were acquired before the onset of ischemia. pH$_i$ was determined from the chemical shift of phosphate relative to phosphocreatine (PCr) with a calibration curve obtained by titrating phosphate in a solution mimicking the intracellular milieu (23).

Statistical analysis. All data are presented as the mean ± SEM for “n” observations. We tested the treatment-time interaction by two-way analysis of variance with repeated measures on time. We used Mauchly’s test to assess the assumption of compound symmetry in each data set. If Mauchly’s test rejected this assumption, the Huyhn-Feldt epsilon correction was used to adjust error degrees of freedom for tests of the treatment-time interaction effect. Differences were judged to be significant when $p < 0.05$ (two-tailed test).

RESULTS

Effects of palmitate on the recovery of cardiac function and efficiency. Figure 1 shows the effects of 1.2 mmol/l palmitate on the recovery of the cardiac work in aerobic and reperfused ischemic hearts. Baseline pre-ischemic values for cardiac work, heart rate, peak systolic pressure, developed pressure, cardiac output, coronary flow and coronary resistance did not differ between glucose + palmitate and glucose hearts (data not shown). Baseline pH$_i$ values in glucose + palmitate and glucose hearts were also not different during the pre-ischemic period (7.16 ± 0.02 and 7.14 ± 0.03, respectively, at the end of 30 min aerobic perfusion). Hearts were subjected to 20 min of global ischemia because this period resulted in a near-complete recovery of cardiac work in glucose heart (83% ± 8% of pre-ischemic values). However, in glucose + palmitate hearts, the recovery of cardiac work was dramatically depressed, returning to only 30% ± 8% of pre-ischemic values by 40 min of reperfusion (Fig. 1). Heart rate (105 ± 26 vs. 238 ± 8 beats/min), peak systolic pressure (68 ± 13 vs. 124 ± 12 mm Hg), cardiac output (25 ± 4 vs. 45 ± 4 ml/min), and coronary flow (9 ± 4 vs. 21 ± 2 ml/min) were all significantly depressed during reperfusion in glucose + palmitate hearts compared with glucose hearts. No difference in coronary resistance was observed during reperfusion in the glucose and glucose + palmitate hearts (4.6 ± 0.7 vs. 6.0 ± 0.8 mm Hg/min/ml). After ischemia, MVO$_2$ in glucose hearts recovered to pre-ischemic values (47 ± 5 vs. 48 ± 4 µmol/g dry wt/min), which was accompanied by a near-complete...
Effects of palmitate on the recovery of pH$_i$. The effect of palmitate on pH$_i$ during ischemia and reperfusion is shown in Figure 1. As expected, pH$_i$ decreased during ischemia, but the extent and rate of this decrease did not differ between the glucose+palmitate and the glucose hearts.

Within the first 3 min of reperfusion there was a 30% recovery of pH$_i$ in both groups, with no differences in the rate of recovery between the glucose and glucose+palmitate groups. However, pH$_i$ in the glucose hearts quickly recovered to pre-ischemic values within the next 2 min of reperfusion, whereas in the glucose+palmitate groups, complete recovery of pH$_i$ required 35 min of reperfusion.

Effects of palmitate on glucose metabolism and H$^+$ production. Cumulative glycolysis and glucose oxidation rates were obtained throughout the entire perfusion period, with rates being linear in both the glucose+palmitate and glucose hearts during the pre-ischemic and post-ischemic periods (Fig. 2). Glycolytic rates in these hearts were lower than rates we have observed in previous studies (13,14). These lower rates are probably related to the lower glucose concentration used in this study (5.5 mmol/l) compared with our previous studies (11 mmol/l). No significant difference in glycolysis rates was observed during reperfusion between the glucose+palmitate and glucose hearts. However, glucose oxidation was significantly depressed in glucose+palmitate hearts compared with glucose hearts. This resulted in a substantial uncoupling of glycolysis from glucose oxidation, resulting in a significantly higher rate of H$^+$ production from glucose metabolism during reperfusion in the glucose+palmitate hearts compared with the glucose hearts (Fig. 2).

Effects of dichloroacetate on the recovery of cardiac function and efficiency. To determine if stimulating glucose oxidation could overcome the detrimental effects of palmitate on cardiac function and pH$_i$ recovery, DCA was added to the perfusate at the onset of reperfusion in the glucose+palmitate hearts (14,18). Dichloroacetate stimulates pyruvate dehydrogenase, the rate-limiting enzyme for glucose oxidation. Figure 3 shows the effects of DCA on the recovery of cardiac work and cardiac efficiency during reperfusion after ischemia. Similar to the results from Figure 1, the recovery of cardiac work was dramatically depressed in hearts perfused with glucose+palmitate, returning to only 38% ± 3% of pre-ischemic values at 40 min of reperfusion. Heart rate (106 ± 22 vs. 234 ± 7 beats/min), peak systolic pressure (70 ± 12 vs. 120 ± 13 mm Hg), cardiac output (26 ± 3 vs. 42 ± 4 ml/min) and coronary flow (10 ± 2 vs. 21 ± 2 ml/min) were also significantly depressed in these hearts during the post-ischemic period, as was cardiac efficiency (Fig. 3). If DCA was present during reperfusion, cardiac work recovered to 74% ± 6% of pre-ischemic values at the end of reperfusion, compared with 38% ± 3% in untreated hearts. Heart rate (226 ± 26 vs. 106 ± 22 beats/min), peak systolic pressure (112 ± 13 vs. 79 ± 12 mm Hg), cardiac output (39 ± 4 vs. 26 ± 3 ml/min) and coronary flow (19 ± 3 vs. 10 ± 2 ml/min) also recovered to a significantly greater extent during reperfusion. No difference in coronary resistance was observed.
During reperfusion in the glucose+palmitate+DCA and glucose+palmitate hearts (5.1 ± 0.5 vs. 4.6 ± 0.8 mm Hg/min/ml⁻¹). At the end of the 40-min reperfusion period, cardiac work in the glucose+palmitate+DCA group recovered to 74 ± 6% of pre-ischemic values. This was accompanied by an overall recovery of MVO₂ to 61 ± 4% of pre-ischemic values. As a result, a complete recovery of cardiac efficiency (cardiac work/O₂ consumed) was observed in the glucose+palmitate+DCA group (to 104 ± 7% of pre-ischemic values) (Fig. 3). This contrasted with the 63% ± 5% recovery of cardiac efficiency in the glucose+palmitate group.

**Effects of dichloroacetate on pHᵢ recovery after ischemia.** The effect of DCA on pHᵢ during the reperfusion period is shown in Figure 3. If DCA was present during reperfusion, a significant increase in the rate of pHᵢ recovery after ischemia was observed. A complete recovery of pHᵢ required 10 min of reperfusion in the glucose+palmitate+DCA hearts, compared with 35 min in the glucose+palmitate hearts.

**Effects of dichloroacetate on glucose metabolism, and H⁺ production from glucose metabolism after ischemia.** Cumulative rates of glycolysis, glucose oxidation and H⁺ production during reperfusion are shown in Figure 4. During reperfusion, DCA selectively increased the rate of glucose oxidation with no significant effect on the rate of glycolysis. During reperfusion, DCA increased glucose oxidation rate to 406% ± 38% of glucose+palmitate alone rates, resulting in a 35 ± 3% decrease in H⁺ production from glycolysis uncoupled from glucose oxidation (Fig. 4). The decrease in H⁺ production during reperfusion in the glucose+palmitate+DCA treated hearts was not as dramatic as that seen in the glucose-alone hearts (Fig. 2). This may explain why the recoveries of cardiac work and cardiac efficiency were slower in the glucose+palmitate+DCA hearts (Fig. 3), compared with the glucose-alone hearts (Fig. 1).

**DISCUSSION**

Proton production and cardiac efficiency. Myocardial energy substrate preference is an important determinant of the ability of cardiac muscle to recovery after an ischemic episode (15,16,23–25). In this study we confirm that a high level of fatty acid (which is seen in most clinically relevant conditions of ischemia) markedly inhibits glucose oxidation during reperfusion of ischemic hearts (14–16). An important novel finding is that the resulting calculated increase in H⁺ production from glycolysis uncoupled from glucose oxidation was accompanied by a delayed recovery of measured pHᵢ during reperfusion. This contributes to a fatty acid-induced decrease in recovery of both mechanical function and cardiac efficiency during reperfusion. The second important finding from this study is that directly stimulating glucose oxidation in fatty-acid perfused hearts (with DCA) accelerates the recovery of pHᵢ during reperfusion, secondary to a decrease in H⁺ production from glucose metabolism. Confirming our previous studies, this stimulation of glucose oxidation was accompanied by a significant improvement in mechanical function and cardiac efficiency during reperfusion (14–16). Of interest is that the beneficial effects of stimulating glucose oxidation occurred during the actual reperfusion period. It is well known that H⁺ accumulation during ischemia is an important contributing factor to ischemic injury (2,26). Our results demonstrate that continued production of H⁺ during the actual reper-

![Figure 3. Effects of dichloroacetate (DCA) on the recovery of cardiac work, cardiac efficiency and pHᵢ of hearts reperfused after ischemia. Values are mean ± SEM of eight glucose+palmitate perfused hearts (closed circles) and eight glucose+palmitate + DCA perfused hearts (closed triangles). Isolated working hearts were subjected to 20 min of global no-flow ischemia and 40 min of aerobic reperfusion. DCA (3 mmol/l) was added immediately before reperfusion. *Significant time-treatment interaction as determined by two-way analysis of variance with repeated measures on time. After application of the Huynh-Feldt correction, p-values for the time-treatment interaction for cardiac work, cardiac efficiency and pHᵢ are 0.004, 0.008 and <0.0001, respectively.](image-url)
Fatty acid inhibition of pH recovery after ischemia. Although \(^{31}\)P-NMR is an effective approach to measure pH in the heart, few studies have used this technique in vitro to measure pH during and after ischemia in hearts perfused with the high levels of fatty acids seen in vivo during and after ischemia. To our knowledge, no previous study has specifically looked at the effects of high levels of fatty acids on rates of pH recovery, nor has pH recovery been directly compared with calculated rates of H\(^+\) production from glycolysis and glucose oxidation. Because metabolic rates are dependent on the work performed by the heart, we developed techniques that allowed measurement of pH in isolated working rat hearts perfused in the presence of a high level of fatty acids (1.2 mmol/l palmitate).

During an episode of global no-flow ischemia, the decrease in pH, in the glucose-perfused hearts was similar to the decrease observed in numerous previous studies (27–29). Of interest is that during ischemia the presence of a high level of fatty acids had no effect on the rate or extent of the decrease in pH. However, during reperfusion the presence of palmitate markedly slowed the rate of pH recovery. Our data suggest that this decreased rate of recovery of pH was the result of a fatty acid-induced increase in H\(^+\) production, as opposed to an alteration in the fate of the H\(^+\) produced. Our data also suggest that this increased H\(^+\) burden contributes to the decrease in cardiac work and cardiac efficiency during reperfusion, because prevention of the fatty acid–induced increase in H\(^+\) production (by stimulating glucose oxidation or by omitting fatty acids) improved the recovery of both cardiac work and cardiac efficiency.

Proton clearance after ischemia. There are at least four different mechanisms that contribute to the recovery of pH from acidosis in the heart: the Na\(^+\)/H\(^+\) exchanger (7), the lactate–H\(^+\) cotransporter (MCT) (30), the vacuolar–H\(^+\) ATPase (31), and the Na\(^+\)/HCO\(_3\)\(^-\) cotransporter (32). The Na\(^+\)/H\(^+\) exchanger is an important determinant of the fate of H\(^+\) during reperfusion, but the clearance of H\(^+\) is associated with the influx of Na\(^+\), which can subsequently lead to the accumulation of intracellular Ca\(^{2+}\) because of alterations in Na\(^+\)/Ca\(^{2+}\) exchange activity. This has led to the development of Na\(^+\)/H\(^+\) exchange inhibitors that are cardioprotective to ischemic hearts (5) by decreasing the amount of ATP necessary to reestablish normal Na\(^+\) and Ca\(^{2+}\) homeostasis (33). The vacuolar–H\(^+\) ATPase may also be an important mechanism by which protons are cleared during reperfusion (34), although clearance of H\(^+\) by this pathway may decrease cardiac efficiency because ATP is required for ATPase activity.

Glucose metabolism as a source of protons in the heart. Although a considerable research effort has concentrated on the fate of H\(^+\) during reperfusion of ischemic hearts, few outside our group have focused on whether H\(^+\) production during reperfusion contributes to ischemic injury. We hypothesize that uncoupled glucose metabolism is an important source of H\(^+\)’s during the actual reperfusion period. As shown in Figure 2, the marked inhibition of glucose
oxidation in the presence of a high level of fatty acid was not accompanied by a similar decrease in glycolytic rates. As a result, glycolysis became further uncoupled from glucose oxidation and continued to be an important source of H⁺ production during the actual reperfusion period. Our data strongly suggest that this is responsible for a slower rate of recovery of pHᵢ during reperfusion.

Although high levels of fatty acids increase H⁺ production in the heart, the detrimental effects of fatty acids may be due to alterations in high energy phosphate production. However, in our study, palmitate did not have any significant effects on ATP, PCR or Pi content measured at the end of ischemia, and levels of these intermediates (ATP, Pi) during reperfusion (data not shown) did not predict the extent of recovery. This lack of correlation between actual levels of high energy phosphates and functional recovery parallels what has been observed previously (35,36).

The use of glucose-insulin-potassium (GIK) solutions has recently received a considerable amount of renewed interest as an approach to treating acute myocardial infarction (38). Although the actual mechanism of GIK was not established, the authors suggest that a lowering of ATP, PCr or Pi content measured at the end of ischemia (ECLA) study showed that GIK can significantly reduce injury by pH paradox. Cardiovasc Res 1993;27:915–24.

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