Substrate-dependent proton load and recovery of stunned hearts during pyruvate dehydrogenase stimulation

JULIAN L. GRIFFIN, LAWRENCE T. WHITE, AND E. DOUGLAS LEWANDOWSKI

Departments of Radiology and Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

Received 2 August 1999; accepted in final form 19 January 2000

Griffin, Julian L., Lawrence T. White, and E. Douglas Lewandowski. Substrate-dependent proton load and recovery of stunned hearts during pyruvate dehydrogenase stimulation. Am J Physiol Heart Circ Physiol 279: H361–H367, 2000.—Stimulation of pyruvate dehydrogenase (PDH) improves functional recovery of postischemic hearts. This study examined the potential for a mechanism mediated by substrate-dependent proton production and intracellular pH. After 20 min of ischemia, isolated rabbit hearts were reperfused with or without 5 mM dichloroacetate (DCA) in the presence of either 5 mM glucose, 5 mM glucose + 2.5 mM lactate, or 5 mM glucose + 2.5 mM pyruvate. DCA inhibits PDH kinase, increasing the proportion of dephosphorylated, active PDH. Unlike pyruvate or glucose alone, lactate + glucose did not support the effects of DCA on the recovery of rate-pressure product (RPP) (without DCA, RPP = 14,000 ± 1,200, n = 6; with DCA, RPP = 13,700 ± 1,800, n = 9). Intracellular pH, from 31P nuclear magnetic resonance spectra, returned to normal within 2.1 min of reperfusion with all substrates except for lactate + glucose + DCA or lactate + DCA, which delayed pH recovery for up to 12 min (at 2.1 min pH = 6.00 ± 0.08, lactate + glucose + DCA; pH = 6.27 ± 0.34, for lactate + DCA). Hearts were also reperfused after 10 min of ischemia with 0.5 mM palmitate + 5 mM DCA and either 2.5 mM pyruvate or 2.5 mM lactate. Again, intracellular pH recovery was delayed in the presence of lactate. PDH activation in the presence of lactate also decreased coupling of oxidative metabolism to mechanical work. These findings have implications for therapeutic use of stimulated carbohydrate oxidation in stunned hearts.

myocardium; reperfusion; nuclear magnetic resonance spectroscopy; dichloroacetate

STIMULATION OF PYRUVATE DEHYDROGENASE (PDH) activity by clinical administration of dichloroacetate (DCA) has been used to support contractility during heart failure (3, 36) and has also been proven effective in preventing myocardial stunning in the isolated heart (17, 20). The mechanism for stimulating PDH with DCA treatment is known, in that DCA is an inhibitor of PDH kinase and thereby acts to increase the proportion of the active form of PDH (34, 35). Whereas PDH is known to be inhibited during early reperfusion (11, 16), what is not known is the mechanism by which countering this reduced activity improves postischemic contractile function.

Earlier considerations suggested that the beneficial effect of stimulated PDH activity is related to increased carbohydrate metabolism (20, 32). However, PDH stimulation is now known to improve contractile function during reperfusion with pyruvate alone in the absence of increased glycolysis (17). Thus the beneficial effects of DCA on the reperfused heart are not limited to the stimulation of glycolysis and glucose metabolism. Intriguingly, whereas the normal myocardium is able to fuel normal contractility during oxidation of lactate, with and without DCA (15), the benefits of DCA on postischemic contractile function are eliminated when lactate is provided as the only substrate (33). Thus the source of pyruvate is important in the response to PDH stimulation.

In this study, we investigated the effect of supplying lactate in combination with glucose, as sources of pyruvate, on the intracellular proton content of the reperfused heart. During an ischemic insult, the concentration of lactate increases in both the blood and the heart (23). Prior studies have examined the effects of DCA on fatty acid oxidation and carbohydrate metabolism during reperfusion (20). However, in this study we investigated why the source of pyruvate appears to be important in supporting the beneficial effects of PDH stimulation. We provide here the first direct measurements of an intracellular pH response to activation of PDH in the heart. We observed that whereas PDH stimulation does not change the substrate dependency of the heart during reperfusion with glucose and lactate, it does delay the recovery of intracellular pH. This delayed recovery seems specific to the presence of lactate and suggests that it produces an additional burden upon recovery of reperfused myocardium.

MATERIALS AND METHODS

Isolated, perfused rabbit heart preparation. Hearts were perfused in retrograde fashion in a nuclear magnetic resonance (NMR) magnet, using previously described methods (15, 17). Use of animals conformed to the guiding principles of the American Physiological Society and Massachusetts...
General Hospital. Hearts were excised from Dutch-belted rabbits (60-75 g) following an intraperitoneal injection of heparin (1,000 IU) and anesthesia (ketamine, 500 mg in 5 ml of saline). Retrograde perfusion was begun with a modified Krebs-Henseleit buffer, at 37°C, and at constant pressure of 100 mmHg. The buffer was phosphate free to enable NMR detection of intracellular phosphate. The recirculated buffer was equilibrated with 95% O₂-5% CO₂. The temperature of the heart was maintained at 37°C. Hearts contracted spontaneously against a fluid-filled intraventricular balloon inflated to an end-diastolic pressure of 5 mmHg and connected to a pressure transducer. Heart rate (HR) and left ventricular developed pressure (LVDP) were continually recorded, and mechanical work was assessed using the rate-pressure product (RPP = HR × LVDP). Oxygen content of the perfusion media and coronary effluent was determined with a blood-gas analyzer for measurement of myocardial oxygen consumption (MV o₂), as previously described (14, 17).

NMR spectroscopy and tissue chemistry. NMR data were collected on a Bruker MSL400 series spectrometer interfaced to a 9.4-Tesla, vertical bore, superconducting magnet (Bruker Instruments, Billerica, MA). 31P spectra were obtained from isolated hearts perfused with a broadband, 20-mm NMR probe (Bruker Instruments) and were recorded at 161 MHz. A 128-scan 31P spectrum was acquired during the preischemic period to assess initial relative energetics and intracellular pH. For measurement of intracellular pH, 31P spectra were acquired, each block being 64 scans and requiring 2.1 min. Detection was continued throughout the periods of ischemia and reperfusion. Free induction decays were Fourier transformed after application of an exponential filter (30 Hz line broadening) to enhance the signal-to-noise ratio. With the use of the phosphocreatine (PCr) peak at 0 parts/million as a chemical shift standard, the chemical shift of the inorganic phosphate resonance was used to calculate intracellular pH (22).

Experimental protocols. Isolated hearts were initially perfused with 5 mM glucose before ischemia to standardize the degree of ischemia so that direct comparison could be made between reperfusion conditions alone. Global perfusion was then interrupted for 20 min to induce ischemic insult.

To study the mechanism of improved postischemic recovery that involves pyruvate oxidation via PDH, the effects of lactate as an alternative source of pyruvate were studied in the presence of glucose, a substrate known to support the respiratory chain whether the retardation in recovery of pH might occur in the postischemic heart compared with carbohydrate reperfusion. Contractile function, intracellular pH, and the final rate of oxygen consumption were monitored in the manners described above.

Statistical analysis. Comparison of results was performed with the Student’s unpaired, two-tailed t-test for comparison between two groups and an ANOVA test of variance for three or more groups. Differences between mean values were considered statistically significant at a probability level of less than 5% (P < 0.05). Results are presented as means ± SE.

RESULTS

Contractile function and oxygen utilization. Whereas PDH stimulation enhanced contractile recovery during reperfusion in the presence of pyruvate and glucose (Table 1), the presence of lactate precluded any beneficial effects on contractile function (Fig. 1). After 20 min of no-flow ischemia, contractile function for isolated hearts reperfused with 5 mM glucose or 2.5 mM pyruvate decreased by ~40% and ~26%, respectively, from the preischemic value (P < 0.005 and P < 0.05, respectively, for RPP at 40 min postinsult compared with initial perfusion RPP by Student’s t-test). The decrease in contractile function recorded following ischemia was prevented by administration of DCA during reperfusion with either 5 mM glucose or 2.5 mM pyruvate (Table 1). However, 5 mM DCA at reperfusion did not aid the recovery in hearts reperfused with 2.5 mM lactate + 5 mM glucose (Fig. 1) (P < 0.05 for RPP at 40 min postinsult in both groups compared with initial perfusion RPP). Contractile function recovery was also impaired in hearts reperfused with 2.5 mM lactate + 5 mM DCA (P < 0.01 for RPP at 40 min postinsult compared with initial perfusion RPP by Student’s t-test).

The respiratory efficiency of contractile performance was decreased in hearts reperfused with lactate and DCA with and without glucose. Oxygen consumption during reperfusion was significantly higher in hearts reperfused with lactate + glucose + DCA compared with lactate + glucose (P < 0.05) (Table 1). This comparison represented a difference in terms of the respiratory efficiency of mechanical function (MV o₂/RPP) (14, 17; Table 1). Coronary flow was not different between any of the groups (ANOVA test of variance) and ranged from 2.4 to 3.6 ml·min⁻¹·g wet tissue wt⁻¹.

Tissue metabolites were extracted from frozen myocardium using 7% perchloric acid. Fractional enrichment with 13C was determined from intr vitro NMR spectroscopy of these tissue extracts, as previously described in detail (6, 21). Lactate content was determined from assays according to Bernt and Bergmeyer (1a).

Recovery of pH during palmitate reperfusion. To investigate whether the retardation in recovery of pH might occur in the postischemic heart, in vivo palmitate, a more physiological substrate, was used. After initial glucose perfusion followed by 10 min of ischemia, hearts were reperfused with 0.5 mM palmitate + 5 mM DCA and either 2.5 mM pyruvate (n = 4) or 2.5 mM lactate (n = 3). The shorter ischemic period was necessary to ensure that the hearts showed adequate functional recovery following ischemia as it is known (9), and we confirmed that palmitate reperfusion significantly reduces the level of contractile function recovery in the postischemic heart compared with carbohydrate reperfusion. Contractile function, intracellular pH, and the final rate of oxygen consumption were monitored in the manners described above.

Comparison of results was performed with the Student’s unpaired, two-tailed t-test for comparison between two groups and an ANOVA test of variance for three or more groups. Differences between mean values were considered statistically significant at a probability level of less than 5% (P < 0.05). Results are presented as means ± SE.

RESULTS

Contractile function and oxygen utilization. Whereas PDH stimulation enhanced contractile recovery during reperfusion in the presence of pyruvate and glucose (Table 1), the presence of lactate precluded any beneficial effects on contractile function (Fig. 1). After 20 min of no-flow ischemia, contractile function for isolated hearts reperfused with 5 mM glucose or 2.5 mM pyruvate decreased by ~40% and ~26%, respectively, from the preischemic value (P < 0.005 and P < 0.05, respectively, for RPP at 40 min postinsult compared with initial perfusion RPP by Student’s t-test). The decrease in contractile function recorded following ischemia was prevented by administration of DCA during reperfusion with either 5 mM glucose or 2.5 mM pyruvate (Table 1). However, 5 mM DCA at reperfusion did not aid the recovery in hearts reperfused with 2.5 mM lactate + 5 mM glucose (Fig. 1) (P < 0.05 for RPP at 40 min postinsult in both groups compared with initial perfusion RPP). Contractile function recovery was also impaired in hearts reperfused with 2.5 mM lactate + 5 mM DCA (P < 0.01 for RPP at 40 min postinsult compared with initial perfusion RPP by Student’s t-test).

The respiratory efficiency of contractile performance was decreased in hearts reperfused with lactate and DCA with and without glucose. Oxygen consumption during reperfusion was significantly higher in hearts reperfused with lactate + glucose + DCA compared with lactate + glucose (P < 0.05) (Table 1). This comparison represented a difference in terms of the respiratory efficiency of mechanical function (MV o₂/RPP) (14, 17; Table 1). Coronary flow was not different between any of the groups (ANOVA test of variance) and ranged from 2.4 to 3.6 ml·min⁻¹·g wet tissue wt⁻¹.
Recovery of intracellular pH during reperfusion. The recovery from acidic pH on reperfusion was delayed during PDH stimulation in the presence of lactate. Intracellular pH was monitored from the chemical shift of the intracellular phosphate resonance in $^{31}$P NMR spectra (Fig. 2). All groups showed a similar decrease in pH during ischemia (lactate + glucose + DCA initial pH = 7.2 ± 0.1, 18.9 min of ischemia pH = 6.0 ± 0.1). Values are means ± SE; n = number of animals. DCA, dichloroacetate; $MV_O^2$, myocardial O$_2$ consumption; RPP, rate-pressure product. Preischemic RPP denotes value during glucose perfusion before ischemia. Values were obtained at 40 min of reperfusion. *P < 0.05 for difference from reperfusion with lactate + glucose + DCA; †P < 0.05, ‡P < 0.01, §P < 0.005 for difference in RPP between initial glucose perfusion and reperfusion. ND, Not determined.

Recovery of intracellular pH during reperfusion. The recovery from acidic pH on reperfusion was delayed during PDH stimulation in the presence of lactate. Intracellular pH was monitored from the chemical shift of the intracellular phosphate resonance in $^{31}$P NMR spectra (Fig. 2). All groups showed a similar decrease in pH during ischemia (lactate + glucose + DCA initial pH = 7.2 ± 0.1, 18.9 min of ischemia pH = 6.0 ± 0.1). Values are means ± SE; n = number of animals. DCA, dichloroacetate; $MV_O^2$, myocardial O$_2$ consumption; RPP, rate-pressure product. Preischemic RPP denotes value during glucose perfusion before ischemia. Values were obtained at 40 min of reperfusion. *P < 0.05 for difference from reperfusion with lactate + glucose + DCA; †P < 0.05, ‡P < 0.01, §P < 0.005 for difference in RPP between initial glucose perfusion and reperfusion. ND, Not determined.

**Table 1. Rate-pressure products and postischemic oxygen consumption**

<table>
<thead>
<tr>
<th>Reperfusion Substrate</th>
<th>Preischemic Rate Pressure Product, mmHg · beats · min$^{-1}$</th>
<th>Postischemic Rate Pressure product, mmHg · beats · min$^{-1}$</th>
<th>Oxygen Consumption, μmol · min$^{-1}$ · g wet wt$^{-1}$</th>
<th>$MV_O^2$/RPP × 10$^4$, μmol · g wet wt$^{-1}$ · mmHg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate + glucose + DCA (n = 9)</td>
<td>20,500 ± 1,700</td>
<td>13,700 ± 1,800†</td>
<td>3.3 ± 0.5</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>Lactate + glucose (n = 6)</td>
<td>20,200 ± 2,000</td>
<td>14,000 ± 1,200†</td>
<td>1.2 ± 0.1*</td>
<td>0.87 ± 0.12*</td>
</tr>
<tr>
<td>Glucose + DCA (n = 5)</td>
<td>20,200 ± 1,000</td>
<td>18,500 ± 1,700a</td>
<td>3.5 ± 0.8</td>
<td>ND</td>
</tr>
<tr>
<td>Pyruvate + DCA (n = 4)</td>
<td>21,900 ± 1,300</td>
<td>19,200 ± 1,600*</td>
<td>3.8 ± 0.6</td>
<td>ND</td>
</tr>
<tr>
<td>Pyruvate (n = 3)</td>
<td>18,400 ± 1,000</td>
<td>13,700 ± 1,800†</td>
<td>2.2 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>Lactate + DCA (n = 4)</td>
<td>21,100 ± 1,000</td>
<td>12,400 ± 2,000‡</td>
<td>3.7 ± 0.5</td>
<td>2.98 ± 0.32</td>
</tr>
<tr>
<td>Lactate (n = 4)</td>
<td>22,300 ± 1,000</td>
<td>12,200 ± 2,200‡</td>
<td>1.1 ± 0.6*</td>
<td>0.91 ± 0.21*</td>
</tr>
<tr>
<td>Glucose (n = 3)</td>
<td>20,800 ± 1,600</td>
<td>12,300 ± 1,100$</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals. DCA, dichloroacetate; MV$_O^2$, myocardial O$_2$ consumption; RPP, rate-pressure product. Preischemic RPP denotes value during glucose perfusion before ischemia. Values were obtained at 40 min of reperfusion. *P < 0.05 for difference from reperfusion with lactate + glucose + DCA; †P < 0.05, ‡P < 0.01, §P < 0.005 for difference in RPP between initial glucose perfusion and reperfusion. ND, Not determined.

**Fig. 1. Recovery of rate-pressure product (RPP) during reperfusion.** Preischemic RPP for the reperfusion group glucose + lactate + dichloroacetate (DCA) is shown at t = 5 min for all three graphs as a comparison. RPP for reperfusion with lactate + glucose and lactate both with and without pyruvate dehydrogenase (PDH) stimulation were significantly decreased compared with reperfusion with either glucose or pyruvate during PDH stimulation for all time points except t = 10 min (P < 0.05). A: ■, pyruvate + DCA; ○, glucose + DCA; ♦, glucose; ◊, pyruvate. B: ○, lactate + glucose + DCA; ■, lactate + glucose. C: ◊, lactate + DCA; ■, lactate.
5.7 ± 0.3, n = 6; lactate + glucose initial pH = 7.0 ± 0.1, 18.9 min of ischemia pH = 5.7 ± 0.2, n = 5). Hearts reperfused with lactate + glucose rapidly returned to normal physiological pH (6.97 ± 0.05 for the temporal average between 21 and 23.1 min, n = 5) (Fig. 3). However, during reperfusion with lactate + glucose + DCA, intracellular pH remained depressed for the first 12 min (At t = 21–23.1 min: lactate + glucose + DCA pH = 6.00 ± 0.08, n = 6; P < 0.05; ANOVA test of variance). This decrease in the rate of recovery of pH did not occur during reperfusion with either glucose, glucose + DCA, pyruvate + DCA, pyruvate or lactate (at t = 21–23.1 min: glucose pH = 7.01 ± 0.03; glu-
cose + DCA pH = 6.99 ± 0.02; pyruvate + DCA = 6.83 ± 0.09; pyruvate = 6.67 ± 0.28; lactate pH = 7.22 ± 0.10). However, a similar retardation in the recovery of pH was detected with lactate + DCA (at t = 21–23.1 min: lactate + DCA pH = 6.27 ± 0.20; P < 0.05 for significant difference from reperfusion with lactate using ANOVA test of variance).

Oxidation of lactate and glucose. Lactate content in the reperfused myocardium was similar in the two groups perfused with lactate and glucose, irrespective of the presence of DCA [lactate concentration: lactate + glucose + DCA = 1.27 ± 0.23 μmol/g wet wt (n = 7), lactate + glucose = 1.16 ± 0.17 μmol/g wet wt (n = 5)].

Two groups of reperfusion in the presence of glucose and lactate, with and without DCA, were also studied to assess the contribution of [3-13C]lactate versus unlabeled glucose to oxidative metabolism. The degree of fractional enrichment for these metabolites was investigated to determine whether PDH stimulation induced a shift in the coupling of oxidative to glycolytic metabolism. No difference was found between the two groups [glucose + lactate + DCA: glutamate enrichment = 0.61 ± 0.10, acetyl-CoA enrichment = 0.85 ± 0.02; glutamate + lactate: glutamate enrichment =
0.54 ± 0.15; acetyl-CoA enrichment = 0.89 ± 0.06 (n = 4 for both groups).

Reperfusion in the presence of palmitate. Contractile function was decreased during reperfusion with palmitate + DCA and either lactate or pyruvate compared with initial contractile function (with lactate; initial RPP = 18,600 ± 400, final RPP = 10,300 ± 1,300; with pyruvate: initial RPP = 18,700 ± 800, final RPP = 13,600 ± 800; P < 0.05 for significant difference between initial and final RPP for both groups). Whereas recovery of contractile function was not significantly increased in the presence of pyruvate compared with lactate, oxygen consumption was decreased (+pyruvate oxygen consumption = 1.77 ± 0.30 µmol O₂ · min⁻¹ · g wet wt⁻¹; + lactate oxygen consumption = 4.61 ± 0.78 µmol O₂ · min⁻¹ · g wet wt⁻¹; P < 0.05). This represented greater coupling of oxidative metabolism to mechanical work during reperfusion in the presence of pyruvate compared with lactate [(MV̇O₂/RPP) × 10⁴ = 1.30 ± 0.14 µmol · g⁻¹ · (bpm · mmHg)⁻¹ for pyruvate + palmitate + DCA; (MV̇O₂/RPP) × 10⁴ = 4.46 ± 0.90 µmol · g wet wt⁻¹ · mmHg⁻¹; P < 0.01 for lactate + palmitate + DCA by Student’s t-test). Despite the decreased acidity induced by the shorter ischemic period in these experiments compared with those discussed above (Figs. 3 and 4), pH recovery was delayed for palmitate + lactate + DCA reperfusion (t = 10.5–12.6 min, pH = 6.2 ± 0.2) compared with palmitate + pyruvate + DCA (t = 10.5–12.6 min, pH = 6.7 ± 0.3; P < 0.05 by Student’s t-test).

DISCUSSION

We investigated whether substrate availability and related proton production mediates the recovery of contractile function in the postischemic heart with or without stimulation of PDH activity. The findings represent the first data to directly demonstrate a measurable response of intracellular pH to stimulation of PDH in the intact, reperfused heart. Lactate was used in conjunction with glucose during reperfusion to investigate the effect the alternative source of pyruvate would have on the known improvement in functional recovery produced by PDH stimulation during reperfusion with glucose. Unlike reperfusion with either glucose or pyruvate during PDH stimulation, the presence of lactate precludes the recovery of contractile function and prevents the rapid restoration of intracellular pH. This effect is specific to reperfusion. Lewandowski et al. (14) have shown previously that lactate perfusion with and without DCA adequately supports contractile function and maintains physiological pH in the nonischemic heart. We further investigated the pH recovery in a number of reperfusion groups to investigate the source of protons, and we found this effect to be specific to the presence of lactate.

Under resting conditions, the plasma lactate concentration of 0.5 mM will not predominate in myocardial metabolism but can increase during periods of intense exercise when plasma lactate increases severalfold (7, 30). Lactate also increases in both the myocardium and blood during periods of hypoxia secondary to failure (2) or ischemia (1, 28). We show in this study that the beneficial effect of stimulating PDH activity reported during reperfusion with glucose or pyruvate in the isolated heart is not apparent during reperfusion with 2.5 mM lactate + 5 mM glucose or 2.5 mM lactate. Previous reports demonstrate neither a correlation between high-energy phosphate content at reperfusion and the beneficial effects of stimulating PDH activity nor an effect of PDH stimulation on high-energy phosphate content in the reperfused heart (3, 14, 16, 17). Thus the current study was focused not on the reactants of phosphoryl group transfer reactions but rather on the role of the metabolic source of pyruvate to fuel the PDH reaction and the consequential recovery of intracellular pH during reperfusion in the presence of stimulated PDH activity.

The beneficial effects of stimulating PDH activity on contractile recovery during glucose or pyruvate metabolism are only apparent if DCA is added for the initial period after the ischemic insult. If DCA is present during ischemia, the myocardium demonstrates metabolism associated with increased loading of lactate into the cytosol (20). During reperfusion with either DCA + lactate + glucose or DCA + lactate, a retardation in recovery of intracellular pH was detected over the period that DCA is thought to act and have a beneficial effect on contractile function. The decrease in the cell’s ability to deal with acidosis may be associated temporarily with DCA-stimulated lactate loading into the cell. Lactate uptake is accompanied by a proton through the monocarboxylic acid transporter (26). This is the same transporter that carries pyruvate (26), and therefore if this effect is caused by the transport of protons into the cell, the pH effect would be observed for both lactate and pyruvate. Furthermore, pyruvate is imported by the cell more readily than lactate and has a similar dissociation constant (pyruvate pKa = 2.39; lactate pKa = 3.08) (4).
Other studies have been performed to suggest that an imbalance between glycolysis and glucose utilization results in an increased H⁺ production and an uncoupling of oxidative metabolism from functional work in the heart (8, 18). This seems unlikely to be the mechanism responsible for the PDH-mediated delay in pH recovery during lactate oxidation. DCA would be expected to increase the coupling between glycolysis and oxidative metabolism. No delay in pH recovery was detected in the groups where increased glycolysis and decreased oxidative metabolism is expected in the absence of DCA (i.e., glucose + lactate, glucose).

13C NMR of myocardial extracts following perfusion with [3-13C]lactate were used to determine the relative enrichments of acetyl-CoA and glutamate. By observing the high-resolution 13C spectra of glutamate, it is possible to determine the fractional enrichment of the tricarboxylic acid cycle from the proportion of isoto-pomers (15, 17, 33). Similar isotopic enrichments of acetyl-CoA and glutamate during reperfusion with lactate + glucose, irrespective of the use of DCA, suggest that stimulating the PDH enzyme complex did not affect the degree of coupling between glycolysis and oxidative metabolism. Whereas total substrate utilization was not measured directly, PDH stimulation also resulted in increased oxygen utilization during reperfusion with lactate + glucose, and this indicates that a commensurate increase in total glycolytic flux occurred to provide the same degree of coupling.

During reperfusion with lactate + glucose + DCA, there was a greater uncoupling of oxidative metabolism from mechanical function as measured by the MV0₂-to-RPP ratio compared with hearts reperfused without DCA. Such an uncoupling was also detected for reperfusion with lactate + DCA and has previously been reported in stunned hearts (16, 17) and during high lactate perfusion alone (27). This increased oxygen demand may be caused by elevated H⁺ load in the presence of DCA with a reduction in oxidative phosphorylation efficiency via mitochondrial Ca²⁺ overload (29). The potential disruption of ion homeostasis may be responsible for negating the beneficial effect of DCA observed during reperfusion with glucose and pyruvate. Uncoupling of oxidative metabolism from contractile function was seen to accompany this delayed recovery in intracellular pH.

The oxidation of lactate to pyruvate by lactate dehydrogenase is accompanied by H⁺ production. By stimulating PDH activity, DCA increases the proportion of lactate oxidized and hence the H⁺ load arising from lactate dehydrogenase activity. This oxidative proton production may slow recovery of pH during reperfusion, leading to increased Na⁺ influx via the H⁺/Na⁺ exchanger, and hence a deleterious influx of Ca²⁺ via the Ca²⁺/Na⁺ exchanger (12, 19, 24, 30–31). Another possibility is that the combined action of DCA and lactate may partially inhibit proton extrusion rather than produce an added proton load. However, there is no report in the pharmacology literature on the myocardium for DCA/lactate blocking any of the ion homoeostasis mechanisms used to restore intracellular pH.

Lactate metabolism also loads the cytosol with NADH via the reverse reaction of lactate dehydrogenase. Under normal metabolic conditions the activity of PDH is closely coupled to the NADH-to-NAD⁺ ratio through PDH kinase (10). The presence of DCA prevents this inhibition of PDH but will not prevent the inhibition of isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase caused by the increase in NADH in the cytosol. DCA-stimulated metabolism of lactate in the normally perfused heart also induces an increase in the pool size of glutamate while maintaining the relative enrichment of the glutamate pool compared with lactate perfusion alone (15). This suggests that the combined effect of lactate and DCA is to stimulate flux across the malate/aspartate shuttle, favoring increased glutamate content at the new equilibrium.

To investigate whether the pH retardation observed during the reperfusion with lactate in the presence of DCA might occur in the postischemic heart in vivo, pH recovery was followed during palmitate + DCA reperfusion with either lactate or pyruvate. Again the recovery of pH was retarded in the presence of lactate but not pyruvate. Whereas DCA failed to produce significant recovery in contractile function during palmitate reperfusion for either substrate mixture, oxygen consumption was significantly increased during the presence of lactate. The retardation in pH recovery detected during lactate and DCA reperfusion is not directly responsible for contractile dysfunction in the postischemic heart, because contractile dysfunction was observed with a number of substrate mixtures without DCA. However, the pH effect did produce an increase in oxygen consumption. This increased oxygen consumption represented a decrease in the oxidative efficiency of the myocardium in terms of contractile force and suggests that stimulated PDH flux in the presence of lactate produces an added burden in the postischemic heart and an uncoupling of functional work from oxidative metabolism.

Other reports of deleterious effects of lactate on cardiac recovery during reperfusion (5) cannot be explained solely by NADH loading of the cytosol. During normal perfusion, the heart readily metabolizes lactate (6, 15). Stunning cannot be explained solely as due to PDH inactivation postischemia, because we have shown that contractile function is not improved during PDH stimulation in the presence of lactate. A third factor, ion homeostasis and subsequent coupling of oxidative metabolism to contractile function, may also be important for the recovery of contractile function during reperfusion with carbohydrate. This effect may be highly dependent on substrate concentration. Using 1 mM DCA, 11 mM glucose, 1.2 mM palmitate, and 0.5 mM lactate, Liu and co-workers (18) measured an improvement in contractile function compared with reperfusion in the absence of DCA. However, the redox load placed on the myocardium in that study was smaller than that experienced during reperfusion with the 2.5 mM lactate and 5 mM glucose used in this
LACTATE OXIDATION, pH, AND POSTISCHEMIC RECOVERY

Koretsune Y and Marban E.

In conclusion, the action of DCA on the reperfused myocardium is substrate dependent, and its beneficial action of stimulating PDH activity is negated by the presence of lactate. The enhanced oxidation of lactate retards the cell’s recovery of physiological pH following an ischemic insult. Therefore, therapeutic protocols to stimulate PDH activity in the postischemic heart should be carefully considered.

This work was supported by National Heart, Lung, and Blood Institute Grant RO1 HL-49244 (to E. D. Lewandowski).

REFERENCES


Downloaded from http://ajpheart.physiology.org/ by 10.220.32.247 on April 20, 2017