Cytosolic redox state mediates postischemic response to pyruvate dehydrogenase stimulation

W.LA urce, J. MICHAEL O'DONNELL, J. JULIAN GRIFFIN, AND E. DOUGLAS LEWANDOWSKI. Cytosolic redox state mediates postischemic response to pyruvate dehydrogenase stimulation. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H626–H634, 1999.—Augmented pyruvate oxidation via pharmacological stimulation of pyruvate dehydrogenase (PDH) during reperfusion has been related to improved recovery of postischemic hearts independent of glycolytic activity. This study examined recovery of postischemic rabbit hearts during activation of PDH with dichloroacetate (DCA) in the presence of lactate, as a source of pyruvate, to determine the response to substrate-dependent changes in cytosolic redox state. After 10 min of ischemia, isolated hearts were reperfused with either 2.5 mM or 0.5 mM pyruvate (Pyr) or 2.5 mM lactate (Lac), with or without 5 mM DCA. 13C-enriched substrates allowed NMR assessment of metabolic perturbations. During normal perfusion, Pyr and Lac supported similar mechanical work. Increasing Pyr oxidation restored postischemic rate-pressure product to 82 ± 4 and 88 ± 6% of preischemic values during reperfusion with 2.5 and 0.5 mM Pyr, respectively, vs. 61 ± 6 and 45 ± 14% for untreated 2.5 and 0.5 mM Pyr, respectively (P < 0.05). In contrast, increasing Lac oxidation did not benefit recovery of RPP in untreated (44 ± 7%) vs. DCA-treated 36 ± 4% hearts. Thus, the benefit of PDH activation for contractile recovery of postischemic hearts is mediated by the source of pyruvate, which also influences cytosolic redox state.

COUNTERING POSTISCHEMIC dysfunction in the “stunned myocardium” with increased carbohydrate oxidation through the pyruvate dehydrogenase (PDH) enzyme complex is now well established in experimental models (14, 15, 23, 20). The activity of PDH is regulated via covalent modification, as the phosphorylated form of the enzyme is rendered inactive by the activity of the PDH kinase enzyme. During the first 10–15 min of reperfusion after transient ischemia in the heart, PDH is predominantly in the phosphorylated, inactive form (7, 12, 22). Pharmacological treatment to inhibit PDH kinase and thereby to increase the percentage of active PDH during reperfusion improves contractile recovery of postischemic hearts. Until recently, this beneficial effect was thought to be directly associated with either enhanced glycolytic flux or the removal of lactate, which would inhibit glycolysis. Recent work in our laboratory (14) has established that PDH stimulation, independent of glycolysis, is effective in improving postischemic contractile recovery. However, the mechanism for this beneficial action on contractile function remains uncertain.

Because the beneficial action of PDH is now known not to result from increased glycolytic flux (14), the search for other potential mechanisms requires further investigation of the link between postischemic contractile function and the action of PDH on its substrate, pyruvate. If improved contractile function during reperfusion is purely dependent on flux through PDH alone, then the source of the substrate pyruvate should be irrelevant in producing the observed effects. Therefore, this study examines the potential for a mechanism involving substrate-dependent changes in the intracellular environment, whereby PDH stimulation may improve recovery of postischemic hearts.

The principal aim of this study was to assess the potential for substrate-dependent differences in cytosolic redox state to influence the effectiveness of PDH stimulation in enhancing recovery of postischemic hearts. The metabolic fate of two sources of pyruvate for mitochondrial oxidation, pyruvate and lactate, was monitored during stimulation of PDH during reperfusion. The results show a distinction in the degree of postischemic recovery afforded by stimulating PDH, based on the metabolic source of pyruvate. This study of the effectiveness of PDH stimulation to improve recovery of postischemic hearts suggests a greater role for lactate and the redox state of the myocyte in influencing postischemic recovery beyond mere substrate availability for oxidative metabolism via PDH.

MATERIALS AND METHODS

Isolated, perfused rabbit heart preparation. Hearts were retrogradely perfused in an NMR magnet, using previously described methods (6, 9, 10, 12–14). Use of animals conformed to the guiding principles of the American Physiological Society and the Massachusetts General Hospital. Hearts were excised from Dutch Belted rabbits (600–750 g) that were given an intraperitoneal injection of heparin (1,000 IU) and then anesthetized with ketamine (45 mg/kg ip) plus xylazine (3 mg/kg ip). Immediately after excision, we immersed the heart in a solution containing (in mM) 20 KCl and 120 NaCl for cardioplegia at 0°C. We attached the aorta to a 100-cm hydrostatic perfusion column and began retrograde perfusion. Hearts were perfused with a modified Krebs-Henseleit buffer at 37°C, containing (in mM) 116 NaCl, 4 KCl, 1.2 CaCl2, 1.2 MgSO4, 1.2 NaH2PO4, and 25 NaHCO3. The buffer was equilibrated with 95% O2–5% CO2 and was recirculated. The initial perfusate supply contained 5 mM glucose in a 2-liter reservoir.

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This perfusate supply was then changed at the start of each 13C enrichment protocol to a 450-ml reservoir of Krebs-Henseleit buffer containing either 2.5 mM [3-13C]pyruvate or 2.5 mM [3-13C]lactate (Isotec, Miamisburg, OH) with no glucose. Temperature of the hearts was maintained at 37°C by a control unit interfaced to the NMR system console and by the temperature of the perfusion medium. Hearts contracted spontaneously against a fluid-filled intraventricular balloon that was connected to a pressure transducer. The balloon was inflated to an end-diastolic pressure of 5 mmHg. Heart rate (HR) and left ventricular developed pressure (LVP) were continually recorded. Mechanical work was assessed by rate-pressure product (RPP = LVP × HR). Oxygen content (P02) of the perfusion medium and coronary effluent was determined with a blood gas analyzer (International Laboratories). Myocardial oxygen consumption (MV02) was determined for each heart from the difference between the P02 of the perfusate at the aortic cannula and the coronary effluent, as previously described (14, 21).

Metabolite assays. Extraction of tissue metabolites was performed on the frozen ventricles using 7% perchloric acid (9, 29). Acid extracts were neutralized, and the contents of glutamate were determined by enzymatic assay (1, 32, 34). Extracts of 1-g tissue samples were lyophilized and then reconstituted in 0.5 ml deuterium oxide for in vitro NMR analysis.

NMR spectroscopy. NMR data were collected on a Bruker MSL400 series spectrometer interfaced to a 9.4-T, vertical-bore superconducting magnet (Bruker Instruments, Billerica, MA). Field homogeneity was adjusted by shimming on proton signal from the sample. 13C spectra were obtained from isolated hearts perfused within a broadband, 20-mm NMR probe (Bruker Instruments) equipped with a proton decoupling coil. In vitro 13C and proton spectra were acquired with a 5-mm probe (Bruker Instruments). Signal intensity of resonance peaks was determined by integration of the area under each peak using NMR-dedicated analysis software (NMRS, Tripos Associates, St. Louis, MO).

13C NMR spectra were obtained sequentially from hearts during perfusion with 13C-enriched substrate to assess potential qualitative changes over time. NMR data were collected and processed as described in previous reports (6, 9, 10, 12–14). 13C spectra were acquired at 101 MHz from intact hearts in 6-min time blocks (152, 45° pulses). Such 13C NMR signals were proton decoupled using power-gated decoupling (0.5–6 W) to avoid sample heating. Before 13C enrichment, a 13C natural abundance signal was acquired from each heart for digital background subtraction from spectra collected during 13C enrichment. NMR signals were processed by exponential filtering with a line broadening of 20 Hz followed by Fourier transformation. Peak assignments were referenced to the known resonance of the exogenous, 13C-enriched substrate and the well-documented glutamate and alanine resonance signals relative to dioxane at 67.4 parts per million. Changes in signal intensities caused by nuclear Overhauser enhancement or relaxation effects were minimal under these pulsing conditions, as previously discussed (3, 13, 17).

In the oxidative metabolism of the mitochondria, the position of the label on both the [3-13C]pyruvate and the [3-13C]lactate contributes to similar placement of the label at the second carbon position (C-2) of acetyl-CoA. After the condensation reaction of the citrate synthase enzyme, the label is positioned at the fourth carbon (C-4) of citrate and then subsequently at the C-4 position of α-ketoglutarate. Efflux of the mitochondrial α-ketoglutarate to the cytosolic compartment enables exchange of label between α-ketoglutarate and the large glutamate pool, via the equilibrium reaction of glutamate-oxaloacetate transaminase. Recycling of C-4-labeled α-ketoglutarate within the tricarboxylic acid (TCA) cycle repositions the label at the C-2 and third carbon (C-3) positions of the intermediates within the second span of the TCA cycle. The result is C-2- and C-3-labeled oxaloacetate, which then enters the citrate synthase reaction. The recycling of label back through the first span of the TCA cycle positions the recycled label to the C-2 and C-3 positions of α-ketoglutarate and subsequently the C-2 and C-3 positions of the NMR-detectable glutamate. This sequence of labeling within the glutamate pool is covered in greater detail in several earlier publications that describe the 13C methodology (2, 6, 7, 17). The resulting multiple labeling of glutamate can be detected in the intact heart, whereas the splitting effects of multiple labeling in the same glutamate molecule can be detected in vitro. The in vitro detection of signal splittings enables the calculation of the fraction of labeled acetyl-CoA (17). This sequence of labeling can also be detected in the intact heart. Sequential 13C spectra from intact, functioning rabbit hearts were examined for the continued oxidation of intracellular pyruvate, from either exogenous pyruvate or lactate.

13C NMR spectra were collected from tissue extracts that were lyophilized and then reconstituted in 0.5 ml of deuterium oxide. The raw 13C NMR signal was collected using 45° excitation pulses with 2-s interpulse delays during broadband proton decoupling. The composite free induction decay (FID) was initially obtained within an 8-kiloword data set, which was then increased to 32 K (zero-filling) to improve digital resolution of the transformed data and processed with a Gaussian filter. From in vitro samples, the multiplet structure within the C-4 glutamate resonance within high-resolution 13C spectra allowed the percentage of labeled acetyl groups entering the citrate synthase reaction of the TCA cycle to be calculated (17) for each heart.

Experimental protocols. Experiments were performed on both normally perfused and postischemic hearts at two different relative levels of cytosolic redox state (NADH/NAD+). Low redox state conditions were induced by perfusion with pyruvate as the only exogenous carbon-based fuel, and high redox state conditions were induced by perfusion with lactate as the only exogenous carbon-based fuel (10, 28).

At the start of each protocol for either normal perfusion or reperfusion, the perfusate supply was switched from the original glucose-containing buffer that was used to support the preparation during experimental setup and preischemic conditions to a similar buffer containing one of two different concentrations, 2.5 mM 99% [3-13C]pyruvate or 2.5 mM 99% [3-13C]lactate, each without glucose. Hearts were perfused for 30 min with 13C-enriched substrate alone (2.5 mM pyruvate, n = 8; lactate, n = 9) or in combination with 5 mM DCA (2.5 mM pyruvate + DCA, n = 7; lactate + DCA, n = 4). DCA inhibited PDH kinase to elevate the level of active, unphosphorylated PDH (30) and stimulate oxidation of either lactate or pyruvate.

In postischemic groups, after the initial perfusion with glucose, hearts were subjected to 10 min of global, zero-flow ischemia followed then by 30-min reperfusion with either pyruvate or lactate alone (2.5 mM 99% [3-13C]pyruvate, n = 10; 2.5 mM [3-13C]lactate, n = 8) or in combination with 5 mM DCA (2.5 mM 99% [3-13C]pyruvate, n = 10; 2.5 mM [3-13C]lactate, n = 13). At the onset of reperfusion, coronary effluent was collected and discarded for the first 2 min to avoid recirculation of accumulated endogenous lactate that was washed out of the myocardium, as determined from previ-
RESULTS

Cardiac function. Mechanical function in normally perfused and reperfused hearts, as assessed by the product of heart rate-developed pressure (RPP), is shown in Fig. 1. Values were consistent with previously reported levels of RPP in the isolated rabbit heart (9, 10, 12, 14) and the in vivo rabbit heart (18, 31, 33). Consistent with previous findings during normal perfusion, lactate supported mechanical work to the same extent and duration as pyruvate, irrespective of the presence of DCA to stimulate oxidation (9, 10). Mean RPP in hearts during normal perfusion with pyruvate was unchanged during 30 min of perfusion, beginning at 24,173 ± 2,171 and ending at 21,779 ± 2,227 mmHg·beats·min⁻¹. During normal perfusion with pyruvate plus DCA, mean RPP also remained unchanged during 30 min of perfusion, beginning at 26,434 ± 3,039 and ending at 24,988 ± 3,877 mmHg·beats·min⁻¹. Mean RPP in hearts perfused with lactate did not change significantly during 30 min of perfusion, beginning at 21,578 ± 1,093 and ending at 19,854 ± 1,377 mmHg·beats·min⁻¹. During normal perfusion with lactate plus DCA, mean RPP also remained unchanged during 30 min of perfusion, beginning at 19,900 ± 2,852 and ending at 17,667 ± 1,250 mmHg·beats·min⁻¹.

As expected from the similarity in work performance among groups that were normally perfused, oxygen consumption (in µmol·min⁻¹·g dry wt⁻¹) at the midpoint of the perfusion period was not different among all groups of normally perfused hearts: pyruvate perfusion, 21 ± 2; pyruvate plus DCA perfusion, 26 ± 2; lactate perfusion, 19 ± 1; lactate plus DCA perfusion, 19 ± 2. Also, no difference in oxygen consumption existed between groups of reperfused hearts. M\textsubscript{O₂} values (in µmol·min⁻¹·g dry wt⁻¹) for each reperfusion group were as follows: pyruvate reperfusion, 21 ± 1; pyruvate plus DCA reperfusion, 26 ± 5; lactate reperfusion, 18 ± 1; lactate plus DCA reperfusion, 20 ± 3. The relatively normal levels of oxygen consumption, despite obviously impaired contractile performance, are consistent with a large body of published work citing the respiratory inefficiency or “oxygen wastage” in the stunned heart (6, 11, 14, 15).

Despite similar oxygen consumption rates and the observation that lactate serves as a source of pyruvate to fuel oxidative metabolism to support function during normal perfusion, postsischemic hearts reperfused with lactate showed poor recovery in comparison to hearts reperfused with pyruvate (see Fig. 1). Among hearts...
subjected to the ischemia and reperfusion protocol, RPP values before the ischemic insult were similar in all groups. Thus no group of hearts, or outstanding individual hearts, displayed impaired function before the ischemic insult that might have otherwise influenced the observed differences in recovery between the experimental groups. After ischemia and reperfusion, DCA in the presence of pyruvate as the sole substrate induced the expected improvement in the recovery of RPP (see Fig. 1). Surprisingly, as is evident in Fig. 1, the beneficial action of DCA was not observed among hearts reperfused with lactate as the sole substrate.

The discrepancy in recovery between DCA-treated, postischemic hearts oxidizing lactate and those oxidizing pyruvate was examined as a potential artifact of reduced availability of pyruvate when lactate served as the source of intracellular pyruvate. To examine this potential effect, separate groups of hearts were also perfused with a lower and more physiological concentration of pyruvate, 0.5 mM (8), with or without DCA. Figure 2 shows the results of this experiment and illustrates the effectiveness of PDH stimulation with DCA to enhance recovery in reperfused hearts, even in the presence of a fivefold reduction in pyruvate concentration.

Substrate metabolism and isotopic enrichment levels. No general qualitative changes in the labeling of metabolites in the heart were observed over the course of the perfusion time, and the evolution of the enrichment patterns in stunned hearts was similar to that reported for normally perfused hearts in previous studies (10, 14). Representative $^{13}$C spectra acquired at steady-state isotope enrichment from hearts reperfused with either 2.5 mM [3-$^{13}$C]pyruvate or 2.5 mM [3-$^{13}$C]lactate are displayed in Fig. 3. Spectra of hearts oxidizing either $^{13}$C-enriched substrate during normal perfusion have already been shown in previous publications (10, 29). No general qualitative differences in spectra existed between normally perfused and reperfused hearts. However, consistent with previous findings in normoxic hearts, both normally perfused and reperfused hearts in this study showed a metabolic response to DCA that differed as to whether the heart was fueled by pyruvate or lactate. These data are described below.

In vitro high-resolution spectra and fractional enrichment measurements indicated distinctions in the use of pyruvate vs. lactate in response to reperfusion with DCA, similar to data from normally perfused hearts that have been previously published (10). A representative sample of such high-resolution data from a heart reperfused with [3-$^{13}$C]lactate is shown in Fig. 4. Hearts perfused or reperfused with pyruvate responded to stimulation of PDH by displaying increased $^{13}$C enrichment of acetyl-CoA entering the TCA cycle. The results of the in vitro $^{13}$C NMR analysis simply demonstrate the contribution of the labeled substrate as a fraction of all substrates, including unlabeled endogenous sources, that were oxidized in the TCA cycle. Figure 5 illustrates this response in hearts perfused or reperfused with pyruvate. The small, unenriched fractions represent endogenous fuel utilization from stores of lipid and glycogen. Also shown in Fig. 5, as previously reported (10), is that DCA treatment eliminated the reduced activation level of PDH in the reperfused heart, counteracting the otherwise reduced fraction of pyruvate that was oxidized by the reperfused hearts.

In contrast, hearts perfused or reperfused with lactate did not show appreciable increases in the $^{13}$C enrichment of acetyl-CoA (Fig. 5). This finding for both normal and postischemic hearts is consistent with a previously reported (10) finding from our laboratory for normally perfused hearts. Instead, lactate induced relatively high levels of acetyl-CoA labeling without DCA treatment in comparison to hearts perfused with pyruvate. Interestingly, both groups of postischemic hearts that were reperfused with lactate showed a significant reduction in the fraction of labeled lactate oxidized in comparison to the corresponding groups of treated and untreated hearts during normal perfusion. This difference is illustrated in Fig. 5. Despite a small reduction in the percentage of lactate being oxidized in the postischemic hearts, in contrast to hearts reperfused with pyruvate, DCA treatment failed to enhance the fraction of the labeled lactate that was being oxidized during reperfusion.

However, both perfused and reperfused hearts oxidizing lactate responded to DCA with an increase in the total glutamate pool, which is shown in Fig. 6. The difference in the glutamate pool in response to DCA stimulation in hearts oxidizing lactate vs. pyruvate has been previously attributed to a shift in metabolite balance due to differences in cytosolic redox state (10, 28). In general, all groups of hearts oxidizing lactate displayed higher levels of tissue glutamate content compared with the corresponding groups of hearts.
oxidizing pyruvate ($P < 0.05$). This general difference in glutamate content is consistent with the notion of a shift toward increased glutamate as a result of the metabolic balance dictated by the higher cytosolic redox state condition in the presence of lactate.

In examining the possibility of an alternate metabolic route contributing to the beneficial effects of DCA, experiments with [3-13C]leucine did not result in any discernible labeling of the glutamate pool irrespective of the presence of DCA. This negative result indicates that the extent of the formation of acetyl-CoA from leucine was not comparable to acetyl-CoA formation from pyruvate or lactate.

Thus, despite providing an adequate oxidizable substrate for PDH, lactate induced a much higher cytosolic redox state that appears to have shifted the metabolic balance of both the normal and reperfusion myocardium. Despite the common metabolic pathways for the oxidation of pyruvate or of lactate as a source of pyruvate, the distinctions in cytosolic redox state that were induced with either substrate are coincident with failure of lactate to support any beneficial effect of stimulating PDH in the postischemic heart.

DISCUSSION

This study examined the substrate-dependent influence of cytosolic redox state and pyruvate oxidation on the recovery of the postischemic, reperfused heart. We observed that the beneficial effects of stimulating the
PDH complex on the recovery of the reperfused heart are eliminated by lactate, which although it is a source of pyruvate, differs with regard to the generation of a high cytosolic redox state (28). As we have already demonstrated that enhanced recovery of the postischemic heart by activation of PDH is independent of glycolytic activity (14), we now show that whereas pyruvate supports this recovery, the source of pyruvate as a fuel does influence the effectiveness of this metabolic perturbation. Interestingly, lower levels of pyruvate are effective in supporting the beneficial effects of DCA on postischemic hearts. However, lactate, as a source of pyruvate, did not support the beneficial effects of DCA. Thus a mechanism for the beneficial effect of PDH stimulation is influenced by cytosolic redox as a consequence of the source of substrate for PDH (10, 28).

Although lactate served as an adequate fuel to support function in the normally perfused myocardium compared with pyruvate, it also demonstrated a lessened ability to support RPP in the reperfused heart. This finding is consistent with previous observations by Schneider and Taegtmeyer (27). As reported for conditions of normal perfusion in a previous study (10), these experiments again show that the labeled, exogenous lactate was more effective than exogenous pyruvate at competing with unlabeled endogenous sources for entry into the TCA cycle in both normal and reperfused conditions. However, hearts reperfused with lactate plus DCA showed no difference in RPP compared with hearts reperfused with lactate in the absence of DCA. This response is clearly not a result of limitations in pyruvate availability, as lowered concentrations of pyruvate [on the order of circulating blood levels in vivo (8)] supported the beneficial actions of DCA.

Whereas it is generally acknowledged that cytosolic NADH content of the myocyte is difficult to accurately measure because of the high NADH content of the mitochondria, Scholz et al. (28) demonstrated that the distinctions in cytosolic redox state can be assessed using glycerol-3-phosphate levels relative to dihydroxyacetone phosphate as an indicator of the cytosolic NADH/NAD+. In this manner, Scholz et al. (28) were able to confirm the distinctly different cytosolic redox...
state conditions that are to be expected between isolated hearts supplied pyruvate vs. hearts supplied lactate. Briefly, the increase in cytosolic lactate concentration establishes a metabolic equilibrium with the reverse flux through lactate dehydrogenase producing a higher baseline level of NADH/NAD⁺ in the cytosol. This lactate-induced increase in cytosolic NADH/NAD⁺ content is detectable as a change in glycerol-3-phosphate concentrations relative to dihydroxyacetone phosphate.

As previously reported (10), induced oxidation of lactate with DCA in the normally perfused heart results in a distinct shift in carbon mass balance between the TCA cycle intermediates and the intracellular glutamate pool. This earlier observation is likely the result of a new equilibrium that is achieved in response to a balance between TCA cycle flux and an elevated rate of malate-aspartate shuttle activity in response to high-cytosolic redox state due to the exogenous lactate. Thus the increase in cytosolic NADH/NAD⁺ increases the net flux across the malate-aspartate shuttle, shifting the equilibrium of the associate metabolite pool toward increased cytosolic glutamate.

A similar condition was observed in this study of the reperfused heart, where glutamate levels became significantly elevated in response to DCA treatment in the presence of lactate. This was not the case for either normally perfused or reperfused hearts oxidizing pyruvate. Therefore, stimulating lactate oxidation in the reperfused heart failed to improve contractile recovery, as it would with pyruvate oxidation, resulting from an apparent relation to elevated cytosolic NADH content.

From these findings, it appears that the beneficial effect of stimulating pyruvate oxidation in postischemic hearts is linked not just to carbon entry into the TCA cycle, but also to the redox state in the cytosol during reperfusion. We have already demonstrated (14) that this improvement in contractile recovery will occur in the absence of greatly elevated glycolytic activity. In this follow-up study, we demonstrate that this response in contractile recovery appears to be mediated by the balance between the cytosolic and mitochondrial redox potential. Thus the beneficial actions of DCA on the reperfused heart may not be a direct consequence of carbohydrate oxidation but rather may involve the homeostasis of cytosolic redox state and the balance between oxidative flux in the mitochondria and nonoxidative metabolism in the cytosol (35). One possibility is that DCA was ineffective during reperfusion with lactate, because the substrate-induced elevation of cytosolic NADH inhibited the recovery of cytosolic NADH during reperfusion, precluding the otherwise beneficial action of PDH stimulation.

The only condition whereby DCA treatment during reperfusion of the isolated heart preparation appears ineffective is during the oxidation of significant quantities of lactate. This study is significant because the mechanism of improved posts ischemic recovery in response to PDH stimulation can be moved away from both the earlier notions of increased glycolytic flux (16, 20) and later considerations that beneficial effects may be more directly linked to the enzyme-activation state itself (14). It is uncertain whether the in vivo heart, where significant concentrations of circulating lactate may arise, will respond to DCA treatment on reperfusion. Whereas DCA has proven ineffective as an agent to promote carbohydrate metabolism (19) during ischemic conditions, the limited oxygen delivery during coronary artery stenosis or ligation may also preclude the advantages of stimulated pyruvate oxidation. Thus the effectiveness of PDH stimulation during reperfusion in vivo has yet to be fully investigated.

This study also examined an alternative metabolic pathway for acetyl-CoA formation that is believed to respond to DCA treatment. Although previous studies (25, 26) have demonstrated radiolabeling of the TCA cycle intermediates via the activity of the branched-chain keto-acid dehydrogenase, our results showed no significant 13C enrichment as a consequence of [3-13C]leucine oxidation, either in the absence or presence of DCA to stimulate this alternate pathway. Whereas the generally negative findings of this particular experiment may also be a consequence of the inherently limited sensitivity of the NMR method, the lack of NMR signal indicates a very low fractional enrichment of <5% as a result of very little oxidation of the leucine.

With the knowledge that differences in glycolytic flux do not account for the effectiveness of enhanced pyruvate oxidation in countering postischemic dysfunction (14), we advanced this investigation further to ask whether the source of pyruvate oxidation made a difference to recovery. The results show that the beneficial effects can be mediated by the cytosolic redox state (NADH/NAD⁺). The role of lactate-induced changes in cytosolic redox state on myocardial function has been assessed during low-flow ischemia (4) but not on the substrate- and PDH-dependent recovery of the reperfused heart. Our experimental plan proved cardiac functional and metabolic responses to lactate and pyruvate as fuel sources of energy production during reperfusion, and it lends new understanding to the link between oxidative intermediary metabolism and contractile activity.

Our results showing enhanced recovery during PDH stimulation with a low concentration of pyruvate indicate that lactate as a source of pyruvate in the myocyte does not support the DCA-induced recovery of postischemic hearts; however, the lower intracellular concentrations of pyruvate produced by lactate are not the limiting factor. The distinct mechanism for recovery between hearts that receive perfusion with pyruvate vs. lactate appears related to the redox state balance between the cytosol and the mitochondria, analogous to a voltage difference across the mitochondrial membrane. Thus, whereas lactate supports normal function and energetics in the normal, well-perfused heart, the change in redox state balance across the mitochondrial membrane, as evidenced by changes in the predominantly cytosolic glutamate pool during stimulated lactate oxidation, during reperfusion with lactate eli-
nated the beneficial effects of stimulating pyruvate oxidation in the postischemic heart.

The significance of this work is that our findings advance previous investigations, which show that pyruvate alone, in the absence of increased glycolysis, can support the beneficial contractile effects of PDH stimulation with DCA (14), and that lactate in the normally perfused heart serves as an adequate source of pyruvate to fuel oxidative energy production (10). Thus the current findings of reduced effectiveness of PDH stimulation in the presence of lactate as a fuel in supporting cardiac function in the stunned myocardium, implicate mechanisms outside of the mitochondria but aside from glycolytic flux, by which PDH stimulation enhances recovery of the postischemic heart. The results also hold relevance to the application of PDH stimulation in the in vivo myocardium as a therapeutic regimen to support postischemic recovery, because of the high levels of regional and circulating lactate that may arise during ischemia and reperfusion in vivo (24, 27).

This work was supported by National Heart, Lung, and Blood Institute Grant R01-HL-49244 (to E. D. Lewandowski) and was done during the tenure of an Established Investigator Award from the American Heart Association to E. D. Lewandowski.

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Received 23 November 1998; accepted in final form 29 March 1999.

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