Dichloroacetate improves cardiac efficiency after ischemia independent of changes in mitochondrial proton leak

MASAYUKI TANIGUCHI, CRAIG WILSON, CHARLENE A. HUNTER, DANIEL J. PEHOWICH, ALEXANDER S. CLANACHAN, and GARY D. LOPASCHUK. Dichloroacetate improves cardiac efficiency after ischemia independent of changes in mitochondrial proton leak. Am J Physiol Heart Circ Physiol 280: H1762–H1769, 2001.—Dichloroacetate (DCA) is a pyruvate dehydrogenase activator that increases cardiac efficiency during reperfusion of ischemic hearts. We determined whether DCA increases efficiency of mitochondrial ATP production by measuring proton leak in mitochondria from isolated working rat hearts subjected to 30 min of ischemia and 60 min of reperfusion. In untreated hearts, cardiac work and efficiency decreased during reperfusion to 26% and 40% of preischemic values, respectively. Membrane potential was significantly lower in mitochondria from reperfused (175.6 ± 2.2 mV) versus aerobic (185.8 ± 3.1 mV) hearts. DCA (1 mM added at reperfusion) improved recovery of cardiac work (1.9-fold) and efficiency (1.5-fold) but had no effect on mitochondrial membrane potential (170.6 ± 2.9 mV). At the maximal attainable membrane potential, O2 consumption (nmol O2·mg−1·min−1) did not differ between untreated or DCA-treated hearts (128.3 ± 7.5 and 120.6 ± 7.6, respectively) but was significantly greater than aerobic hearts (76.6 ± 7.6). During reperfusion, DCA increased glucose oxidation 2.5-fold and decreased H+ production from glucose metabolism to 53% of untreated hearts. Because H+ production decreases cardiac efficiency, we suggest that DCA increases cardiac efficiency during reperfusion of ischemic hearts by increasing the efficiency of ATP use and not by increasing the efficiency of ATP production.

Cardiac efficiency can also potentially be decreased after ischemia as a result of uncoupled or inefficient ATP production from oxidative phosphorylation. Even under nonpathological conditions the coupling of electron transport to phosphorylation is variably incomplete in that transport-driven O2 consumption can occur in the absence of phosphorylation. This gives rise to a backflow of protons into the mitochondrial matrix, a condition termed proton leak (2). As a consequence, the O2 consumed to drive the futile cycle of proton flux across the mitochondrial membrane is not used for ATP synthesis and the phosphorylation/oxygen ratio declines. Mitochondria isolated from ischemic hearts have been shown to have an increased rate of proton leak (5, 6), which in the face of the hypoxic conditions that define ischemia could significantly impair ATP production. The lack of sufficient ATP would not only affect cardiac recovery but would also have a significant impact on the necrotic and apoptotic pathways initiated by oxidative stress.

ALTERATIONS IN ENERGY METABOLISM that occur both during and after ischemia are important determinants of ischemic injury in the heart. During reperfusion of reversibly injured ischemic hearts, mitochondrial tricarboxylic acid (TCA) activity and mitochondrial respiration can rapidly recover, whereas contractile function remains depressed (1, 16, 20, 22). This results in a dramatic impairment in cardiac efficiency (measured as cardiac work per O2 consumed or cardiac work per tricarboxylic acid acetyl-CoA production) during reperfusion (16, 22). Whether this decrease in cardiac efficiency is due to a decrease in the efficiency of converting ATP into cardiac work or to a decrease in the efficiency of ATP production from mitochondrial respiration has not been unequivocally determined.

After acute myocardial infarction or cardiac bypass surgery, plasma levels of fatty acids are markedly elevated (19). This contributes to a high rate of fatty acid oxidation during reperfusion, even though contractile function can be depressed (1, 15, 16, 18, 22). These high rates of fatty acid oxidation markedly inhibit glucose oxidation, resulting in significant uncoupling between glycolysis and glucose oxidation during reperfusion (16, 22). This results in an increase in the production of protons from glycolytically derived ATP (16, 20, 22). Accumulation of these protons may contribute to the accumulation of Na+ and Ca2+ in the postischemic period through an increase in Na+/H+ exchange and Na+/Ca2+ exchange activity (11, 14, 29). This can result in a decrease in cardiac efficiency during the reperfusion period, which can be prevented by inhibition of the Na+/H+ exchanger (11).

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Cardiac efficiency can be improved during reperfusion of ischemic hearts by either inhibiting fatty acid oxidation (18) or by directly stimulating glucose oxidation (16, 22). Dichloroacetate (DCA) can overcome fatty acid inhibition of glucose oxidation by stimulating the pyruvate dehydrogenase complex, the rate-limiting enzyme for glucose oxidation (30). Stimulation of glucose oxidation with DCA not only enhances contractile function during reperfusion (21, 24, 27) but it also significantly improves cardiac efficiency (16, 22). We (16, 22) have previously suggested that the beneficial effects of DCA on cardiac efficiency occur as a result of a decrease in $H^+$ production from glycolysis uncoupled from glucose oxidation. However, it has not been determined whether the beneficial effects of DCA result in increases in efficiency of oxidative phosphorylation, increases in the efficiency of ATP use by the myocardium, or a combination of both.

In this study we determined whether DCA decreases mitochondrial proton leak in hearts reperfused after ischemia. Isolated working rat hearts perfused with a high level of fatty acid (1.2 mM palmitate) were subjected to a 30-min period of global no-flow ischemia, followed by 60 min of aerobic perfusion. The effects of DCA on cardiac work and efficiency were measured during reperfusion. As well, using mitochondria isolated from perfused hearts, the effects of DCA on membrane potential and mitochondrial $O_2$ consumption were measured. Our results suggest that the increase in postischemic efficiency seen with DCA is not due to changes in mitochondrial proton leak, but rather to a decrease in $H^+$ production from glucose metabolism during reperfusion, with a resultant increase in the efficiency in ATP use.

METHODS

Heart perfusions. All animal procedures were approved by the University of Alberta Health Sciences Animal Welfare Committee and conformed to the guidelines of the Canadian Council on Animal Care. Hearts were removed from anesthetized rats and cannulated for working heart perfusions as described previously (18, 21). In brief, male Sprague-Dawley rats (300-350 g, Charles River Laboratories; Montreal, Quebec) were anesthetized with pentobarbital sodium, and the hearts quickly excised and immersed in ice-cold Krebs-Henseleit solution. The aorta was cannulated and a retrograde perfusion at 37°C and 60 mmHg of hydrostatic pressure was initiated. Subsequently, the pulmonary artery and the left atrium were cannulated. After a 10-min equilibration period, hearts were switched to the working mode by clamping the left atrial inflow line from the Langendorff reservoir and opening the left atrial inflow and aortic outflow lines. Oxygenated perfusate was delivered into the left atrium at a preload pressure of 11.5 mmHg. Perfusion was conducted at a preload pressure of 80 mmHg. All working hearts were perfused with Krebs-Henseleit solution containing 2.5 mM free Ca$^{2+}$, 11 mM glucose, 100 $\mu$U/ml insulin, 1.2 mM palmitate, and 3% BSA (fraction V, Boehringer-Mannheim). Palmitate was bound to the albumin as described previously (28).

Spontaneously beating hearts were used throughout the studies. Heart rate, and aortic pressure were measured with a Gould P21 pressure transducer in the aortic outflow line. Cardiac output and aortic flow were measured with Transonic ultrasound flow probes in the preload and afterload lines, respectively. Coronary flow was calculated as the difference between cardiac output and aortic flow. The $O_2$ content in the perfusate was measured using a YSI micro-oxygen electrode in a line originating from the cannulated pulmonary artery. Myocardial $O_2$ consumption ($MVO_2$) was calculated according to the Fick principle, using coronary flow rates and the arteriovenous difference in perfusate $O_2$ concentration. Cardiac work was calculated as the product of systolic pressure and cardiac output. Cardiac efficiency was defined as the ratio of cardiac work to $MVO_2$, and as the ratio of cardiac work to TCA cycle activity (the total rate of acetyl-CoA production for TCA cycle).

Experimental protocols. Working hearts were initially perfused for a 30-min period under aerobic conditions. Global ischemia was then introduced by clamping both the left atrial inflow and aortic outflow lines. After 30 min of global ischemia, the left atrial and aortic flows were restored, and the hearts were reperfused for 60 min under aerobic conditions. Randomly, hearts were either left untreated or treated with DCA (1 mM) added to the circulating buffer after the ischemic episode and preceding the aerobic reperfusion.

Measurement of glucose oxidation and palmitate oxidation. Glucose and palmitate oxidation were measured simultaneously by perfusing hearts with $[U^{-14}C]$glucose and $[9, 10-^{3}H]$palmitate as described previously (28). The total myocardial $^{14}CO_2$ and $^{3}H_2$O production were determined at 10-min intervals during both the initial aerobic perfusion period and during the 60-min period of reperfusion. Glucose oxidation was determined by quantitative measurement of $^{14}CO_2$ production, whereas palmitate oxidation was determined by quantitative collection of $^{3}H_2$O production by the heart (28).

The rate of acetyl-CoA production for the TCA cycle was calculated assuming 2 acetyl-CoA were produced from glucose oxidation and 8 acetyl-CoA from palmitate oxidation.

Preparation of mitochondria. Immediately after either the initial preischemic aerobic perfusion or at the end of the aerobic reperfusion period, the hearts were perfused with 20 ml of ice-cold buffer containing 220 mM mannitol, 70 mM sucrose, 5 mM Tris-HCl, 1 mM EGTA, and 0.5 mg/ml nase (protease), pH 7.4. Hearts were then trimmed of atria and blood vessels, minced with sharp scissors in fresh buffer, and incubated for 10 min on ice. Minced tissue was then washed twice with fresh buffer with added fatty-acid-free BSA (5 mg/ml) and homogenized for 10 s with a Polytron homogenizer at half-maximum speed followed by a 15-s rest period and a second 10-s homogenization. The homogenate was centrifuged at 600 g for 3 min and the resultant supernatant was filtered through four layers of cheesecloth. The pellet was resuspended and centrifuged again at 600 g for 3 min. Pooled supernatants were then centrifuged at 12,000 g for 15 min. The final mitochondrial pellet was washed twice in fresh buffer and then suspended in 300 mM sucrose and 10 mM Tris-HCl, pH 7.4.

Measurement of mitochondrial proton leak. Characteristically, the rate of proton leak across the inner mitochondrial membrane increases disproportionately with increasing membrane potential ($\Delta\psi$) with the maximum leak rate occurring in the absence of phosphorylation and ion transport (state 4 respiration) (2). Thus under these conditions the simultaneous measurement of $O_2$ consumption and $\Delta\psi$ as the respiratory rate is varied provides an estimate of the rate of proton leak.

In the present study, mitochondrial $O_2$ consumption and membrane potential were measured at 30°C in 2.5 ml of
Effects of dichloroacetate (DCA) on the recovery of mechanical function of hearts during reperfusion after 30 min of global ischemia. Figure 1 shows the effect of DCA on the recovery of cardiac work in hearts reperfused after 30 min of no-flow ischemia. Cardiac work remained depressed in untreated hearts after ischemia, with cardiac work returning to only 20% of preischemic values (preischemic cardiac work was $62.2 \pm 2.7$ ml·min$^{-1}$·mmHg$^{-1}$·10$^{-3}$). Addition of DCA (1 mM) at reperfusion resulted in a significant improvement in cardiac work (Fig. 1), with hearts recovering to 38.2% of preischemic values. As shown in Table 1, developed pressure, heart rate, times peak systolic pressure, cardiac output, aortic flow, and coronary flow were all significantly depressed during reperfusion in both control and DCA-treated hearts. However, the presence of DCA during reperfusion significantly improved the recovery of developed pressure, heart rate times peak systolic pressure, cardiac output, aortic flow, and coronary flow compared with untreated hearts.

During reperfusion of untreated hearts, MV$\text{O}_2$ recovered to a greater extent than cardiac work resulting in significant decrease in cardiac efficiency (Table 2). In DCA-treated hearts, the increase in cardiac work during reperfusion compared with untreated hearts (Fig. 1) was not accompanied by a parallel increase in MV$\text{O}_2$ (Table 2). This resulted in a significantly greater recovery of cardiac efficiency in DCA-treated hearts compared with untreated hearts.

Effects of DCA on glucose and palmitate oxidation of hearts during reperfusion of ischemic hearts. Rates of glucose oxidation and fatty acid oxidation before and after ischemia are shown in Fig. 2, A and B, respectively. As expected during the initial aerobic period, glucose and palmitate oxidation rates were similar in untreated and DCA-treated hearts, because DCA was not present during the preischemic period. During reperfusion, glucose oxidation rates recovered to preischemic levels in untreated hearts, whereas palmitate oxidation...
oxidation rates were significantly lower during reperfusion. Glucose oxidation rates during reperfusion in DCA-treated hearts were significantly increased compared with both preischemic values and postischemic control values (Fig. 2A). In contrast, the presence of DCA had no effect on rates of palmitate oxidation during reperfusion (Fig. 2B).

The contribution of glucose oxidation to the TCA cycle activity is shown in Fig. 3. Under these conditions of a high level of fatty acid, palmitate oxidation provided 80–85% of the TCA cycle acetyl-CoA during the initial aerobic period. During reperfusion of untreated hearts, palmitate oxidation also provided over 85% of the TCA cycle acetyl-CoA. In DCA-treated hearts the contribution of glucose oxidation to TCA acetyl-CoA increased from 15% to 46%.

Effects of DCA on mitochondrial O\textsubscript{2} consumption and membrane potential in mitochondria isolated from aerobic and reperfused ischemic hearts. Mitochondria were isolated from preischemic and postischemic hearts to determine whether the improvement in cardiac efficiency seen during reperfusion in DCA-treated hearts was related to an improvement in the efficiency of ATP production (i.e., decreased proton leak). State 4 mitochondrial proton leak is graphically shown in Fig. 4 for preischemic untreated hearts and postischemic untreated and DCA-treated hearts. Mitochondrial membrane potential was lower in both postischemic untreated and DCA-treated hearts relative to mitochondria isolated from preischemic untreated hearts (Fig. 4).

Maximum obtainable membrane potential and O\textsubscript{2} consumption are shown in Fig. 5, A and B, respectively.

Table 2. Effect of dichloroacetate on oxygen consumption and cardiac efficiency in hearts reperfused following 30 min of global no-flow ischemia

<table>
<thead>
<tr>
<th>Parameter Measured</th>
<th>Untreated</th>
<th>Dichloroacetate</th>
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<tbody>
<tr>
<td></td>
<td>Preischemia</td>
<td>Postischemia</td>
</tr>
<tr>
<td>Oxygen consumption, (\mu\text{mol} \text{O}_2\cdot\text{min}^{-1}\cdot\text{g dry wt}^{-1})</td>
<td>52.6 ± 4.2</td>
<td>28.8 ± 5.1*</td>
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<tr>
<td>Cardiac efficiency, (\text{ml}\cdot\text{mmHg}\cdot\mu\text{mol} \text{O}_2\times 10^{-2})</td>
<td>1.26 ± 0.11</td>
<td>0.51 ± 0.1*</td>
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Values are means ± SE of 15 untreated and 8 DCA treated hearts. Hearts were subjected to 30 min of aerobic perfusion, 30 min of global no-flow ischemia, and 60 min of aerobic reperfusion. DCA, when present, was added at the onset of reperfusion. Preischemic values were determined before the onset of ischemia, whereas postischemic values were determined at the end of the reperfusion period. *Significantly different from preischemic values.
Mitochondrial membrane potential did not differ between mitochondria isolated from reperfused postischemic untreated and DCA-treated hearts, but were lower than preischemic hearts (Fig. 5A). At the maximal attainable membrane potential, mitochondrial O₂ consumption was greater in mitochondria from both postischemic control and DCA-treated hearts compared with mitochondria from preischemic control hearts (Fig. 5B). Respiratory control ratios were not different between preischemic and postischemic mitochondrial preparations, i.e., 3.9 ± 0.2 and 4.01 ± 0.3, respectively.

**DISCUSSION**

During reperfusion of the heart after ischemia, MV˙O₂ and TCA cycle activity rapidly recover even though mechanical work remains depressed (1, 11, 15, 16). A similar finding was observed in this study, with cardiac efficiency (work/O₂ consumed) being significantly depressed during reperfusion of ischemic hearts compared with the preischemic period. We also demonstrate that a decrease in the efficiency of ATP synthesis by mitochondrial oxidative phosphorylation is a contributing factor to this decrease in efficiency. An increase in O₂ consumption at maximal membrane potentials was observed in mitochondria isolated from reperfused ischemic hearts, indicating an increase in mitochondrial proton leak. This decreases the efficiency of coupling O₂ consumption to ATP synthesis, resulting in a decrease in cardiac efficiency. However, a second mechanism by which cardiac efficiency can be decreased after ischemia is due to a decrease in the efficiency of converting ATP into contractile work. If ATP is directed toward reestablishing intracellular ion gradients during reperfusion, less ATP is available for contractile purposes (1, 11, 16). Alterations in energy substrate preference during reperfusion is one mechanism by which ATP utilization can be altered. We recently demonstrated that stimulation of glucose oxidation with DCA decreases H⁺ production in the heart, resulting in an increase in cardiac efficiency (16). In this study we also observed an increase in cardiac efficiency.
efficiency whether DCA was present during reperfusion. An additional finding was that the increase in contractile function and cardiac efficiency during reperfusion in DCA-treated hearts was not accompanied by a significant decrease in mitochondrial proton leak. As a result, our data demonstrate that stimulation of glucose oxidation improves cardiac efficiency by improving the efficiency of coupling ATP production to cardiac work, and not to an improved efficiency of synthesizing ATP.

Benzi and Lerch (1) have shown in reperfused ischemic rat hearts that ruthenium red inhibition of mitochondrial Ca$^{2+}$ transport and sarcoplasmic reticulum Ca$^{2+}$ release can reestablish a normal relation between contractile function and oxidative metabolism. This suggests the involvement of intracellular Ca$^{2+}$ in the mechanisms underlying the dissociation between contractile function and oxidative metabolism during reperfusion. One possible source of this Ca$^{2+}$ is from altered Na$^+$/Ca$^{2+}$ exchange activity, which can occur secondary to Na$^+$ accumulation during and after ischemia due to an acceleration of Na$^+$/H$^+$ exchange activity (29). In support of this, Hata et al. (11) show that inhibition of the Na$^+$/H$^+$ exchanger decreases the O$_2$ cost of contractility in acidic hearts. These authors speculated that inhibition of Na$^+$/H$^+$ exchange in acidic hearts decreases Na$^+$ and Ca$^{2+}$ accumulation, lessening the requirement for ATP for processes involved in the reestablishment of ion homeostasis.

In this and previous studies, addition of DCA at reperfusion significantly improved functional recovery of hearts after ischemia (1, 14, 21, 24, 27). The beneficial effects of DCA are due to a stimulation of pyruvate dehydrogenase (PDH) activity, the rate-limiting enzyme for glucose oxidation. PDH activity is controlled by reversible phosphorylation, in which the phosphorylated form is inactive. PDH kinase and PDH phosphatase are responsible for phosphorylation and dephosphorylation of the complex, respectively. DCA inhibits PDH kinase, thus increasing the proportion of PDH in its active form (30). A recent study by Morton et al. (25) has shown that DCA also inhibits degradation of the E1 $\alpha$-subunit of PDH, further contributing to an increase in PDH complex activity.

Although DCA stimulates PDH and glucose oxidation in the postischemic heart, the reason why this increases contractile function and cardiac efficiency has not been unequivocally established. We have previously attributed the beneficial effects of PDH stimulation during reperfusion to an improved coupling between glycolysis and glucose oxidation (14, 21, 24). High levels of fatty acids seen in the clinical setting of ischemia (19), inhibit glucose oxidation resulting in an imbalance between glycolysis and glucose oxidation (14, 18). However, when glycolysis is coupled to glucose oxidation, two H$^+$ are consumed in the overall oxidation of pyruvate to CO$_2$. As a result, if glycolysis is coupled to glucose oxidation, the net production of H$^+$ is zero (21).

Because DCA improves the coupling of glycolysis to glucose oxidation (16, 21), we speculate that DCA reduces H$^+$ production during reperfusion by improving the balance between glycolysis and glucose oxidation. Although we did not directly measure proton production in this study, we recently showed (17) that addition of DCA at reperfusion causes a significant decrease in H$^+$ production from glucose metabolism. This was associated with an increase in the rate of intracellular pH recovery compared with untreated hearts, as well as in improvement in cardiac efficiency (17).

An alternative mechanism by which DCA may exert its beneficial effects is by influencing mitochondrial proton leak. Proton leak occurs when proton motive force is consumed without ATP synthesis (2, 6). The precise mechanisms responsible for proton leak have not yet been fully elucidated. One possibility is that proton leak occurs due to a failure of the respiratory chain to pump protons at a high protonmotive force, termed slip reactions (2, 6). An alternate explanation involves the presence of natural uncoupling proteins found in the inner mitochondrial membrane. These proteins dissipate the mitochondrial proton gradient producing heat instead of ATP. Although these proteins were originally thought to exist only in brown adipose tissue, novel uncoupling proteins (UCP-2 and UCP-3) have recently been cloned, which are expressed in most tissues studied in humans and rodents (4). The exact role of these proteins on mitochondrial proton leak has not been determined. However, the most supported theory to date is that changes in proton conductance across the mitochondrial membrane occur and these changes are strongly dependent on proton motive force (2).

Decreasing mitochondrial proton leak decreases O$_2$ consumption and increases the efficiency of ATP production. Previous studies by Borutaite et al. (2, 3) have demonstrated that hearts subjected to 45 min of ischemia have increased mitochondrial proton leak. Our results showed a similar trend after 30 min of ischemia, but these differences did not reach statistical significance. However, our results during reperfusion show an increase in O$_2$ consumption at maximal membrane potential in reperfused hearts, indicating a significant increase in mitochondrial proton leak. Although the reason for the increase in mitochondrial proton leak is not clear, it is likely that damage to the mitochondria that occurs during ischemia or reperfusion causes an increase in permeability of the inner mitochondrial membrane. Borutaite et al. (3) suggest that high levels of fatty acids or some other lipophilic factor may accumulate in the mitochondria during ischemia or reperfusion. Another possibility is that
elevated levels of Ca^{2+} in the mitochondria inhibit complex II of the respiratory chain (3). Regardless of the mechanisms responsible for increased mitochondrial proton leak, our results demonstrate that DCA does not alter the increase in proton leak seen in state 4 respiration during reperfusion. As a result, it is unlikely that the beneficial effects of DCA on cardiac efficiency are due to a decrease in proton leak.

Regardless of whether alterations in proton leak are involved, our results suggest that a stimulation of glucose oxidation is a potential therapeutic approach to treating ischemic heart disease. Other potential metabolic approaches to increasing glucose oxidation and decreasing proton production, involve lowering fatty acids with either nicotinic acid (8), β-blockers, or the use of glucose-insulin-potassium infusions (10). By reducing the mitochondrial acetyl-CoA/CoA ratio, L-carnitine, and its analog propionyl L-carnitine have been shown to increase glucose oxidation and benefit myocardial function (7). An increase in tissue L-carnitine content has also been shown to lessen ischemic injury, in both experimental and clinical studies (12, 26). Because high fatty acid oxidation rates markedly decrease glucose oxidation, another approach to increasing glucose oxidation is to inhibit fatty acid oxidation. Pharmacological compounds in this group include the agents trimetazidine and ranolazine, both of which inhibit fatty acid oxidation (13, 23), and carnitine palmitoyltransferase-1 inhibitors such as etomoxir (18). All of these fatty acid oxidation inhibitors stimulate glucose oxidation and improve reperfusion recovery of ischemic hearts (20).

In conclusion, cardiac efficiency is markedly depressed during reperfusion of ischemic hearts. DCA added at reperfusion results in both an increase in contractile function and a significant increase in cardiac efficiency. This increase in cardiac efficiency is not due to an increase in the efficiency of ATP production due to a decrease in mitochondrial proton leak. Rather, combined with previous results, we suggest that the beneficial effects of DCA on cardiac efficiency are due to an increase in the efficiency of ATP utilization due to a lower requirement of ATP to correct H^{+}-induced Na^{+} and Ca^{2+} accumulation.

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