Facilitating Postischemic Reduction of Cerebral Lactate in Rats

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Background and Purpose: Dichloroacetate facilitates a decrease in brain lactate during reperfusion after incomplete ischemia. This study examined the possible activation of pyruvate dehydrogenase enzyme by dichloroacetate to explain this effect. Because the duration of ischemia and hyperglycemia exacerbate ischemic brain damage, the effect of both of these factors on lactate reduction with and without dichloroacetate treatment after ischemia also was explored.

Methods: The two-vessel occlusion and controlled blood loss model of stroke was applied to anesthetized rats. Samples of cerebral cortex were analyzed for lactate by enzyme fluorometry and for pyruvate dehydrogenase activity by radioassay.

Results: Treatment with dichloroacetate produced no significant stimulation of pyruvate dehydrogenase after ischemia. When the duration of ischemia was increased or 50% glucose was infused before ischemia, brain lactate was significantly higher ($p<0.01$, Duncan's test). After 30 minutes of ischemia, treatment with a low dose of dichloroacetate (25 mg/kg) improved the reduction in lactate ($p<0.01$, Duncan's test).

Conclusions: These results indicate that although dichloroacetate reduces brain lactate after cerebral ischemia, the mechanism of action does not involve dichloroacetate's known ability to stimulate pyruvate dehydrogenase. However, these data support the use of dichloroacetate to lower cerebral lactate, especially in cases where ischemia is ≥30 minutes in duration. They also suggest that early restoration and maintenance of perfusion after ischemia and discontinuing the use of 50% glucose before impending ischemia likewise would facilitate reduction of postischemic brain lactate. (Stroke 1992;23:1145–1153)

KEY WORDS • cerebral ischemia • glucose • lactates • rats

Clinically there is a direct correlation between high blood glucose and poor neurological outcome after stroke$^1$ and cardiac arrest.$^2$ Even moderate$^1$ and low$^1$ levels of hyperglycemia can exacerbate brain damage and edema from ischemic insults. Experimentally, an increase in morbidity and mortality with hyperglycemia has been linked to excessive lactate and/or accompanying acidosis that results from the anaerobic metabolism of glucose during ischemia.$^5,6$

The mechanism by which increased lactate and/or acidosis exacerbates the cellular pathology of cerebral ischemia is unknown.$^7$ Lactosis and/or acidosis may affect cells directly$^8$ and/or potentiate other mechanisms of cellular dysfunction, e.g., by increasing intracellular calcium concentration,$^9$ enhancing lipid peroxidation,$^{10}$ and/or suppressing or irreversibly damaging synaptic transmission.$^{11}$ Pathologically, high levels of lactate adversely affect biochemical and physiological recovery from ischemia in the rat$^{12}$ and severely damage neurons, glia, and capillary endothelial cells.$^{13,14}$ This pannecrosis of brain tissue promotes the development of secondary brain damage,$^{15}$ edema,$^{16}$ and seizures,$^{5,17}$ all of which contribute to the increased vulnerability of the injured brain to secondary insults.$^{18}$

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At present, nothing can be done to lessen the accumulation of lactate during a stroke, acute hypotensive incident, cardiac arrest, or head trauma. However, it is possible to lower lactate more quickly after the ischemic attack, thereby limiting the exacerbation of the primary insult and/or the development of secondary brain damage.

Sodium dichloroacetate (DCA) has been used clinically to reduce serum lactate.$^19$ By stimulating pyruvate dehydrogenase enzyme complex (PDHC), DCA increases the metabolism of pyruvate through the tricarboxylic acid cycle, thus promoting the catabolism of lactate.$^{20}$ Because DCA activated PDHC both in vitro$^{21–23}$ and in vivo$^{21,24}$ in brain and because in one study there was the coincident stimulation of PDHC and decrease in lactate in the brains of the same rats,$^{24}$ the activation of PDHC was proposed as the mechanism by which DCA lowers lactate more expediently after ischemia of the brain$^{25,26}$ and spinal cord.$^{27,28}$ The present study tested that hypothesis for brain.

After ischemia, the level of lactate in the brain is influenced not only by the rate of lactate metabolism$^{29}$

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but by how quickly lactate is removed from the brain. Glucose availability and duration of ischemia are potentially important influences on both lactate metabolism and reduction as well as on the effectiveness of DCA in treating lactosis of ischemia. Therefore, a second goal of this study was to evaluate the reduction of brain lactate with and without DCA treatment as a function of the duration of ischemia and the glycemic state of the rat.

Reportedly, insulin-induced hypoglycemia activates and glucose-infusion hyperglycemia depresses PDHC activity in the brains of normal animals. During ischemia, PDHC activity is maintained or elevated; during reperfusion, it is depressed. The evaluation of the effects of ischemia on PDHC activity and the relation of glycemia to PDHC activity under our experimental conditions was the third goal of this study.

Materials and Methods

Animals, Experimental Design, and Experimental Groups

Adult male Wistar rats (n=180; weight, 363±4 g) were randomly assigned to three experimental groups. The rationale for these groupings was as follows.

Analysis of factors affecting reduction in brain lactate. To analyze the effect of duration of ischemia on brain lactate reduction independent of variations in blood glucose, rats in the first experimental group (n=123) were fed ad libitum and divided into five subgroups, one for each duration of ischemia (i.e., 0, 10, 15, 20, and 30 minutes) followed by either 0 or 30 minutes of reperfusion. To determine the effect of DCA on brain lactate, rats in the 30-minute reperfusion subgroups were treated intravenously 3 minutes after ischemia with either DCA (25 mg/kg) or an equivalent volume of saline intravenously. Glucose or saline was infused during the first 8 minutes of the 15 minutes immediately before ischemia.

Analysis of factors affecting PDHC activity. To determine the effect of glycemia on PDHC activity, tissue was analyzed from fed or fasted rats that had been treated with 50% glucose or saline intravenously as described above (see Figure 1 for number of rats in each group).

To analyze the effects of ischemia with and without DCA treatment on PDHC activity, tissue was sampled from fed rats described above that were exposed to 0 (n=5), 15 (n=5), or 30 (n=6) minutes of ischemia or 30 minutes of ischemia with reperfusion with (n=7) and without (n=8) DCA treatment.

The third group of rats (n=6) was used to evaluate PDHC assay methodology and DCA dose. To test PDHC assay methodology, we used a dose previously reported to increase PDHC activity in rat brain. Each rat received an intraperitoneal injection of DCA (250 mg/kg) (n=3) or an equivalent volume of saline (2 ml/kg) (n=3) four times over 2 hours before anesthesia and euthanasia by in situ freezing of the brain.

To compare the effect of DCA on PDHC activity at the higher intraperitoneal dose to that at the lower intravenous dose used in our previous in vivo studies, data from rats injected intraperitoneally were compared with data from sham-ischemic fed rats injected intravenously with DCA (25 mg/kg) or an equal amount of saline (2 ml/kg) as a control as described above.

The protocol for this study was approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Experimental Model

A model of partial global ischemia was slightly modified from that described previously. Briefly, each rat was weighed; anesthetized with halothane (3%) in nitrous oxide (70% N₂O:30% O₂); intubated or tracheostomized for continued ventilation on halothane (0.6%) in nitrous oxide; prepared for ligation of the common carotid arteries by isolating those vessels; operated on for the placement of the right internal jugular as well as tail and/or femoral vessel cannulas for the recording of mean arterial blood pressure (MABP), the exchange and sampling of blood, and the administration of glucose, DCA, and control fluids; prepared for in situ freezing of the brain by opening the scalp; and
instrumented with needle electrodes for electroencephalogram recording.

After this study had begun, the fact that brain hypothermia for 25-30°C or after ischemia may be protective against irreparable brain damage of ischemia was published. Although brain temperature does not affect brain lactate, as a control we needed to verify that brain temperature in these experiments was within an appropriate range. Therefore, a needle probe was placed in the temporalis muscle for recording brain temperature in a representative group of rats, i.e., those given 25% glucose (n=23).

After surgery, halothane was discontinued, heparin (0.05 ml) was injected intravenously, anectine (0.1 ml of a 2-mg/ml dose) was given approximately every 15 minutes throughout the experiment, and the rat was equilibrated for 30 minutes. For ischemia, each rat was bled to an MABP of 80 mm Hg, both carotids were clamped, and the rat was bled further to and maintained at an MABP of 40±10 mm Hg for the duration of ischemia. At the end of ischemia, clamps were removed and shed blood reinjected. Blood pressure, hematocrit, glucose, pH, gases, temperature, and electroencephalogram were monitored before, in the middle of, and at the end of equilibration, ischemia, and reperfusion. Because it was difficult to sample from the arterial line at the end of ischemia because of low MABP, at that time blood was sampled from the venous line.

Arterial pH, oxygen and carbon dioxide tensions (P02 and PCo2, respectively), bicarbonate ion (HCO3-), and glucose were determined as described in previous studies. Briefly, blood gases were measured using a blood gas machine (IL 513 Blood Gas Analyzer, Fisher Scientific Co., Pittsburgh, Pa.). Blood glucose was determined using an Accu-Check BG Monitor (Biodynamics Co., Boehringer Mannheim Corp., Indianapolis, Ind.) because it provides an expedient method for measuring relative differences in circulating glucose in a large number of blood samples from a large number of rats.

Tissue Preparation

Rats were euthanized by in situ freezing of the brain. Brains were processed and sampled bilaterally for tissue from the frontal/parietal motor cortex as described in our previous studies.

Lactate Analysis

Samples of brain (5-10 mg) were homogenized with hydrochloric acid–methanol and extracted. Lactate was determined in these extracts by the enzymatic fluorometric methods of Lowry and Passonneau.

Pyruvate Dehydrogenase Activity Analysis

Brain tissue was prepared for analysis as described by Baudry and coworkers.22 Samples (>25 mg) were homogenized in 1 ml of a cold solution containing 25 mM 1,4-piperazinediethanesulfonic acid (Pipes) and 1 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.2) and sonicated for 10 seconds at 2-second intervals on ice. To prevent a change in the activity of PDHC during the preparation and assay procedures, 200 μl of an inhibiting solution containing 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N′,N″,N‴-tetraacetic acid (EGTA), 2.5 mM DCA, and 8 mM sodium fluoride (pH 7.2) were added to 200 μl of the homogenate.

The resulting solution was assayed for PDHC activity as described by Sheu and coworkers. Briefly, hyamine hydroxide (250 μl) was placed in each scintillation vial. An assay solution (200 μl) was added to a small vented test tube that was fitted to a serum stopper and placed inside the scintillation vial, which was then sealed with the same rubber stopper. This scintillation vial was placed in a 37°C shaking water bath, and 50 μl of tissue homogenate was injected through the stopper into the small test tube inside each vial. The reaction was started by injecting 4 μl of 25 mM [1-14C]pyruvate (1 mCi/ mmol), giving a final concentration of 0.5 mM pyruvate. The vials were incubated for 20 minutes, at which time 50 μl of 10% trichloroacetic acid was injected to stop the reaction. Vials were incubated for an additional 20 minutes, after which 12 ml of scintillation fluid was added to the vials containing hyamine hydroxide, and radioactivity was counted with a Beckman scintillation counter. Protein content was measured in duplicate samples according to the method of Bradford. Dilution during extraction and assay resulted in a total protein of at least 1.128 mg/ml in the solution assayed. This was well above the recommended value of 0.2 mg/ml, below which many enzymes become unstable because of surface denaturation.

Statistical Analysis

Means of parametric data were compared using analysis of variance (STATPAK, Northeast Analytical, Inc., Portland, Ore.). If there were significant differences among means, Duncan’s multiple range test (STATPAK) was applied to determine which groups were significantly different. Nonparametric data (i.e., percentages) were analyzed using the Kruskal-Wallis test (STATPAK), and correlation coefficients were determined with the Hewlett-Packard C-41 statistical package curve-fitting program.

Results

Physiological Variables

Some rats (n=23) were excluded from analysis for the following reasons: 1) eight died during the procedure; 2) nine had a P02 <70 mm Hg and/or an MABP that did not recover to >70% of control levels at the end of reperfusion; 3) three were excluded because of equipment failure; and 4) three fed rats, not given glucose, had brain lactates that after 20 (n=1) or 30 (n=2) minutes of ischemia and 30 minutes of recirculation were higher than the mean±SEM value for a similar period of ischemia alone. Based on our previous studies showing that some rats do not reperfuse after this type of insult, an elevation in brain lactate that is maintained after 30 minutes of supposed recirculation indicates that no reperfusion of the brain has occurred. Because the rats are treated after ischemia and because recirculation is necessary for DCA to be effective, those animals are not included in this analysis.

Body temperature (mean±SEM) for each experimental period was as follows: preischemia, 37.13±0.08°C; at the end of ischemia, 37.14±0.08°C; and, at the end of reperfusion, 37.31±0.08°C. These values were similar to those recorded in previous stud-
There were no significant differences in body temperature between groups in which there were differences in brain lactate, indicating that body temperature was adequately controlled for the purposes of these experiments.

Brain temperature (mean±SEM) in fasted rats given 25% glucose (n=23) averaged 36.91±0.11°C before ischemia, decreased significantly (p<0.01, Duncan’s test) to 35.57±0.17°C during ischemia, and by the end of reperfusion increased to preischemic levels in both saline-(36.59±0.29°C) and DCA-treated (37.21±0.22°C) groups. In spite of the decrease during ischemia, use of a heating lamp maintained the brain temperature close to the 36°C considered normothermic for these experiments.

In control rats (n=31), MABP, hematocrit, blood gases, HCO₃⁻, and pH did not change significantly during either sham ischemia or recirculation. As expected, in ischemic rats (n=126), MABP and venous hematocrit, Po₂, HCO₃⁻, and pH were decreased and Pco₂ increased from sham-ischemic controls. After recirculation all values except HCO₃⁻ in the 20- and 30-minute untreated and the 10-, 20-, and 30-minute DCA-treated rats returned to preischemic levels. (Values for these parameters are available on request.)

**Blood Glucose**

Before ischemia, blood glucose in fasted rats was directly proportional to the amount of glucose administered (R²=0.96). Ten minutes of ischemia had no effect on those levels (Table 1). However, after 30 minutes of reperfusion, glucose was reduced from preischemic and postischemic values in both saline- and DCA-treated rats (Table 1).

In fed rats, ischemia of any duration raised blood glucose significantly to a similar level (Table 1). After reperfusion with and without DCA treatment, glucose was decreased significantly from immediate postischemic levels but remained significantly elevated from preischemic levels except for the 15-minute ischemic rats (Table 1).

**Brain Lactate**

Before ischemia there was no difference among lactate levels in fed or fasted rats with or without glucose (Table 2). As determined for 30-minute sham-ischemic rats, there was no effect of surgical preparation and length of anesthesia on lactate levels (mean±SEM for sham-treated rats, 3.37±0.20 μmol/g; for DCA-treated rats, 2.60±0.16 μmol/g; see Table 2 to compare with preischemic levels).

In fed rats, there were no significant differences among the groups with various durations of ischemia (Table 2). Reperfusion with saline and with DCA reduced all levels significantly below immediate postischemic levels except for saline treatment of the 20-minute ischemic rats (Table 2). When compared with saline, treatment of ischemic rats with DCA resulted in significantly lower lactate levels at the end of reperfusion only in 30-minute ischemic rats (Table 2). Lactate levels in sham-ischemic and either saline- or DCA-treated (2.6±0.2 μmol/g) rats were not significantly different from those in nonischemic control (3.0±0.3 μmol/g) rats.

During reperfusion, the percent decrease in lactate was inversely related to the duration of ischemia (R²=0.88). Treatment with DCA improved the percentage reduced by as much as 46% in the 30-minute ischemic rats. For all durations of ischemia, the percent improvement with DCA treatment was directly proportional to the duration of ischemia (R²=1.00).

The increase in lactate after 10 minutes of ischemia in fasted rats was directly proportional to the amount of glucose administered (R²=0.94) (Table 2). Although still significantly increased above preischemic levels, after 30 minutes of reperfusion lactate decreased significantly from immediate postischemic levels (Table 2). Levels in saline-treated rats did not differ from those in

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**Table 1. Blood Glucose for Fed and Fasted Rats**

<table>
<thead>
<tr>
<th>Experimental state (n)</th>
<th>Duration of ischemia (min)</th>
<th>Preischemia (μmol/g)</th>
<th>Postischemia (μmol/g)</th>
<th>After reperfusion (30 min) +saline (μmol/g)</th>
<th>After reperfusion (30 min) +DCA (μmol/g)</th>
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</thead>
<tbody>
<tr>
<td>Fasted +saline (6)</td>
<td>10</td>
<td>84±2.6</td>
<td>75±19.7</td>
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<td>...</td>
</tr>
<tr>
<td>Fasted +25% glucose</td>
<td>10</td>
<td>311±13.1</td>
<td>301±13.8</td>
<td>159±9.3††</td>
<td>172±13.6††</td>
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<tr>
<td>Fasted +50% glucose</td>
<td>10</td>
<td>763±44.2</td>
<td>673±34.7</td>
<td>394±31.2††</td>
<td>397±18.2††</td>
</tr>
<tr>
<td>Fed (40)</td>
<td>10</td>
<td>153±5.5</td>
<td>353±17.5</td>
<td>221±20.1††</td>
<td>227±15.0††</td>
</tr>
<tr>
<td>Fed (11)</td>
<td>15</td>
<td>153±15.0</td>
<td>405±30.1</td>
<td>174±2.1†</td>
<td>221±13.7††</td>
</tr>
<tr>
<td>Fed (15)</td>
<td>20</td>
<td>150±7.9</td>
<td>368±20.7</td>
<td>243±22.2††</td>
<td>232±16.4††</td>
</tr>
<tr>
<td>Fed (22)</td>
<td>30</td>
<td>142±7.5</td>
<td>366±13.2</td>
<td>243±22.4††</td>
<td>209±30.4††</td>
</tr>
</tbody>
</table>

DCA, dichloroacetate. Values are mean±SEM (mg/dl). All preischemic, postischemic, and reperfusion values for fed rats at different durations of ischemia were not significantly different.

* p<0.01 (Duncan’s test) different from preischemic value; † p<0.01 (Duncan’s) different from postischemic value.
TABLE 2. Brain Lactate for Fed and Fasted Rats

<table>
<thead>
<tr>
<th>Experimental state</th>
<th>Duration of ischemia (min)</th>
<th>Preischemia</th>
<th>Postischemia</th>
<th>After reperfusion (30 min)</th>
<th>After reperfusion (30 min) + DCA (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted+saline (6)</td>
<td>10</td>
<td>2.7±0.2</td>
<td>13.7±1.6*</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3)</td>
<td>(3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted +25% glucose (23)</td>
<td>10</td>
<td>...</td>
<td>25.7±1.5†</td>
<td>7.6±1.5‡</td>
<td>8.5±1.1‡</td>
</tr>
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<td></td>
<td></td>
<td>(5)</td>
<td>(8)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>Fasted +50% glucose (17)</td>
<td>10</td>
<td>2.8±0.1</td>
<td>30.2±1.6$§</td>
<td>21.3±5.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td>(5)</td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>Fed (40)</td>
<td>10</td>
<td>3.0±0.3‡</td>
<td>19.0±0.7*</td>
<td>4.2±0.7†</td>
<td>4.1±0.6‡</td>
</tr>
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<td></td>
<td>(5)</td>
<td>(11)</td>
<td>(14)</td>
<td></td>
</tr>
<tr>
<td>Fed (11)</td>
<td>15</td>
<td>3.0±0.3‡</td>
<td>19.9±2.5*</td>
<td>5.9±1.3‡</td>
<td>4.1±1.1‡</td>
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<td>(11)</td>
<td>(3)</td>
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<tr>
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<td>19.9±4.1*</td>
<td>11.2±3.0</td>
<td>7.8±2.8‡</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>(4)</td>
<td>(7)</td>
<td></td>
</tr>
<tr>
<td>Fed (21)</td>
<td>30</td>
<td>3.0±0.3‡</td>
<td>23.6±2.1*</td>
<td>15.0±1.8§</td>
<td>7.9±1.7$§</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5)</td>
<td>(6)</td>
<td>(8)</td>
<td></td>
</tr>
</tbody>
</table>

DCA, dichloroacetate. Values are mean±SEM (μmol/g). All preischemic and postischemic values for fed rats were not significantly different.

*p<0.01 (Duncan's test) different from preischemic value; tp<0.01 (Duncan's) different from fasted+saline value; tp<0.01 (Duncan's) different from postischemic value; §p<0.05 (Duncan's) different from fasted+25% glucose value; ||p<0.05 different from postischemic value; $value for fed rats euthanized before ischemia; #p<0.01 (Duncan's) different from saline-treated value.

rats given DCA (Table 2). The percent decrease in lactate was considerably less in those rats given 50% glucose when compared with those given 25%. Treatment with DCA improved that rate in rats given 25% glucose by <0.5%, but in rats given 50% glucose, the decrease in lactate was improved to 59%.

Pyruvate Dehydrogenase Activity

Pyruvate dehydrogenase activity was lower in rats fasted overnight (Figure 1). Administration of 50% glucose significantly increased PDHC activity in fed but not in fasted rats (Figure 1).

In rats given the higher DCA dose, PDHC activity (mean±SEM) was significantly increased over control (6.24±0.48 nmol/mg per minute versus 3.00±0.22 nmol/mg per minute). The lower intravenous dose also increased PDHC activity, i.e., from 2.30±0.39 to 3.02±0.38 nmol/mg per minute, but not significantly from control values. When calculated as percent increase over control, there was a significant difference between the responses in PDHC activity to the two doses (p=0.0339, Kruskal-Wallis) (Figure 2).

In fed rats, after 15 minutes of ischemia there was a significant increase in PDHC activity over preischemic levels (Figure 3). After 30 minutes of ischemia, PDHC activity was not significantly different from preischemic levels. At the end of recirculation, activity had significantly decreased from preischemic and immediately postischemic levels by the end of reperfusion (1.27±0.22 nmol/mg per minute), a result that was not affected by treatment with DCA (1.31±0.24 nmol/mg per minute). Plotting brain lactate along the same time course demonstrates that lactate after 30 minutes of ischemia is elevated and with DCA treatment decreases significantly without a concomitant increase in PDHC activity (Figure 3).

**Discussion**

As in previous studies, treatment with DCA facilitated reduction in brain lactate during reperfusion after 30 minutes of ischemia.25-26,34-36 This effect of DCA on lactate in tissue has been demonstrated for normal brain24 as well as for pathological tissues, including brain25 and spinal cord27 of ischemic animals after recirculation. Although it has been speculated that DCA mediates this effect by stimulating PDHC, this has not been established in any of these tissues.

The data of this study demonstrate that DCA activation of PDHC is not the mechanism involved in DCA effect on brain lactate. Treatment with DCA at a dose
that stimulated activity in nonischemic brain and lowered lactate during reperfusion after 30 minutes of ischemia had no effect on PDHC activity after ischemia. Another laboratory has shown that even a higher dose of DCA does not stimulate PDHC activity in the ischemic brain. However, in contrast to our study, those investigators did not see an effect of DCA on brain lactate, probably because of an insufficient duration of ischemia, i.e., 15 minutes instead of 30 minutes. In our study, DCA treatment after 15 minutes of ischemia also did not improve lactate reduction. By contrast, DCA treatment after 30 minutes of ischemia did lower brain lactate more quickly, but it did not activate PDHC. That is, we did not observe an inverse relation between PDHC activity and lactate levels in the same tissue. Therefore, the data of this study do not support the hypothesis that brain lactate reduction with DCA is due to PDHC activation.

If lactate is not lowered because of an increase in PDHC activity, how does DCA mediate the decrease in lactate? One possible mechanism is that a DCA-mediated decrease in arterial lactate would enhance the removal of lactate from the ischemic cell, which would constitute an indirect effect of DCA on the transport of lactate. Our results of in vitro experiments using a transformed human astrocytoma cell line (UC11-MG) suggest that DCA also may have a direct effect on the transport of lactate. We determined that treatment with DCA inhibits high lactate/low pH-induced swelling of human astrocytoma cells by 50%. This inhibition by DCA is independent of activation of PDHC by DCA; instead, it is the result of DCA's competitive inhibition of lactate accumulation by those cells. Data from that in vitro and the present in vivo experiments suggest that the decrease in lactate with DCA treatment in vivo may be related to DCA's effect on the transport of lactate rather than its effect on PDHC activity.

Data in this study also indicate that clinically controllable factors, i.e., nutritional state of the subject, blood glucose, and duration of ischemia, significantly affect the amount of lactate generated during ischemia and reduction in brain lactate during reperfusion after ischemia. In this study preischemic stores and/or availability of blood glucose during ischemia were correlated directly to the amount of lactate generated during ischemia and to the amount remaining after reperfusion. In addition, this study demonstrated that the effectiveness of DCA in reducing tissue lactate increases with the duration of ischemia independent of the amount of lactate that accumulates during ischemia. These data support the suggestions that 1) under the condition of acute or anticipated cerebral ischemia, a patient's blood glucose should be closely monitored and controlled and 2) shortening the ischemic period, e.g., early reestablishment of blood flow, perhaps with a thrombolytic agent, will facilitate lactate reduction.

What are potential mechanisms by which hyperglycemia and duration of ischemia could interfere with lactate reduction? Although DCA does not affect lactate by altering PDHC activity directly, changes in PDHC activity induced by variations in nutrition, glyce mia, ischemia, and/or reperfusion could affect lactate metabolism indirectly.

Fasting has been reported to decrease the proportion of PDHC activity in a variety of tissues. We also observed a decrease in PDHC activity in the brains of rats that had been fasted. Glucose had no stimulatory effect on PDHC activity in those rats. This may be dose dependent because at a higher dose, Cardell and coworkers reported an increase in PDHC activity in fasted rats infused with glucose to maintain normoglycemia. In contrast to fasted rats, fed rats given glucose evidenced an increase in PDHC activity. This is similar to glucose stimulation of PDHC activity in rat adipocytes. These results demonstrate the importance of nutritional state as it affects glycemia in determining the effect of DCA on PDHC activity.

As in other studies, our data showed that a short period of ischemia, e.g., 15 minutes, stimulated an increase in PDHC activity that was decreased during reperfusion. However, we also demonstrated that when ischemia was extended to 30 minutes there was a decrease in PDHC activity that after 30 minutes of reperfusion was depressed even further. In another study, this depression after 15 minutes of ischemia lasted for up to 6 hours. Pretreatment with DCA at the higher intraperitoneal dose in that study or postischemic treatment with the lower intravenous dose in the present study did not affect the depressed PDHC activity after 15 or 30 minutes of recirculation, respectively. The changes described above for PDHC activity in relation to nutritional state, glycemia, ischemia, and reperfusion are not inversely proportional to those for ischemia,
lactate. This indicates that changes in PDHC due to those factors are not related to the changes in lactate.

A more logical reason for limited reduction of lactate with hyperglycemia and increased duration of ischemia may be based on an increased production of lactate during reperfusion under these conditions. It has been observed that high levels of blood glucose correlate with decreased tissue perfusion after ischemia. A decrease in blood flow after ischemia would constitute an increased duration of ischemia and promote secondary anoxia. If glucose was still available to the tissue, anaerobic glycolysis would promote an increased buildup of lactate during this low-flow reperfusion. A decrease in reperfusion also would limit the amount of lactate that could be cleared from the brain by the circulatory system. Therefore, decreased blood flow during reperfusion would produce a new increase in tissue lactate that would appear as a limited reduction of brain lactate during the recirculation period.

These results demonstrate that DCA treatment, glucose infusion, and duration of ischemia are important influences on lactate reduction in brain tissue during reperfusion. Controlling the amount of lactate generated by limiting substrate availability before ischemia is impossible for strokes, severe hypotension, cardiac arrest, or head trauma when they are not anticipated events. However, the mechanism of damage to astrocytes and capillary endothelium that occurs after ischemia can be caused by elevated lactate. Therefore, if a decrease in brain lactate can be facilitated after ischemia, subsequent brain damage could be less severe. The results of our study suggest that this potentially beneficial increase in the net reduction of lactate from the brain after ischemia can be mediated by 1) DCA treatment that may facilitate the cellular transport of lactate, 2) limiting blood glucose, i.e., substrate availability, and 3) promoting and maintaining reperfusion after the ischemic episode.

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References

Lactate and acidosis are products of anaerobic metabolism that are now thought to be important participants in tissue injury produced by cerebral ischemia/reperfusion in various models of stroke. Evidence is accumulating, as discussed in the article by Dimlich and Nielsen, indicating that enhancement or suppression of anaerobic glycolysis (by elevated or lowered blood glucose levels) produces increased or reduced degrees, respectively, of ischemia-elicited neuronal tissue injury and lactate production. The lowering of tissue lactate release by dichloroacetate also produces protection against ischemic injury. Since the maintenance of energy metabolism appears to be central to neuronal cell viability and function, these effects of blood glucose levels suggest that processes in addition to ATP production appear to be important contributors to cerebral ischemic injury. The data in the study by Dimlich and Nielsen on brain lactate levels in rat cerebral ischemia further demonstrate the modulation of this metabolite by the duration of ischemia, blood glucose levels, and dichloroacetate. In addition, evidence is provided that dichloroacetate appears to function through a mechanism(s) other than the stimulation of pyruvate dehydrogenase. An alternative mechanism suggested by these authors is that dichloroacetate may attenuate lactate release through inhibition of its transport; however, the actual alternative mechanism(s) of action of dichloroacetate remains to be established.

A major component of ischemia-elicited neuronal tissue injury appears to involve oxygen radical–related processes. It has been suggested that the release of iron, a catalyst for hydroxyl radical formation and lipid peroxidation, is caused by products of anaerobic metabolism such as acidosis. Lactate, however, has recently been shown to cause brain tissue injury, particularly to astrocytes and endothelium, by a mechanism(s) that appears independent of the actions of anoxia alone.

A recent study has demonstrated that lactate caused markedly enhanced free radical formation compared with acidosis in brain tissue homogenates, suggesting that lactate could be involved in the generation of oxygen radicals or the modulation of free radical–related processes. A concept that has not been previously explored is the possibility that lactate (and other products of anaerobic metabolism) might enhance oxygen radical production through increased cytosolic levels of NADH by the lactate dehydrogenase reaction. In a detailed study of cellular sources of oxygen radical formation in hepatic tissue, Boveris and coworkers identified a microsomal electron transport chain utilizing an NADH cytochrome b5 reductase and cytochrome b5 as a site of cellular oxygen radical formation. In cultured human umbilical vein endothelium, NADH can serve as a cosubstrate in the cytochrome b5 reductase activity in vascular smooth muscle that appears to be the major detectable source of superoxide anion formation in this tissue.

Our laboratory has recently found a similar NADH oxidoreductase activity in vascular smooth muscle that appears to be the major detectable source of superoxide anion formation in this tissue. In addition, NADH can serve as a cosubstrate in the peroxidase reaction of cyclooxygenase, resulting in the production of superoxide anion. Cyclooxygenase has been demonstrated to be a major source of oxygen radical production in the cerebral microcirculation and in cultured human umbilical vein endothelium. In addition to producing injury, oxygen radicals may participate in causing vasodilation, which would contribute to the removal of toxic products of anaerobic metabolism.
Facilitating postischemic reduction of cerebral lactate in rats.
R V Dimlich and M M Nielsen

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