

# Pyruvate Dehydrogenase Complex: Metabolic Link to Ischemic Brain Injury and Target of Oxidative Stress

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The mammalian pyruvate dehydrogenase complex (PDHC) is a mitochondrial matrix enzyme complex (greater than 7 million Daltons) that catalyzes the oxidative decarboxylation of pyruvate to form acetyl CoA, nicotinamide adenine dinucleotide (the reduced form, NADH), and CO<sub>2</sub>. This reaction constitutes the bridge between anaerobic and aerobic cerebral energy metabolism. PDHC enzyme activity and immunoreactivity are lost in selectively vulnerable neurons after cerebral ischemia and reperfusion. Evidence from experiments carried out *in vitro* suggests that reperfusion-dependent loss of activity is caused by oxidative protein modifications. Impaired enzyme activity may explain the reduced cerebral glucose and oxygen consumption that occurs after cerebral ischemia. This hypothesis is supported by the hyperoxidation of mitochondrial electron transport chain components and NAD(H) that occurs during reperfusion, indicating that NADH production, rather than utilization, is rate limiting. Additional support comes from the findings that immediate posts ischemic administration of acetyl-L-carnitine both reduces brain lactate/pyruvate ratios and improves neurologic outcome after cardiac arrest in animals. As acetyl-L-carnitine is converted to acetyl CoA, the product of the PDHC reaction, it follows that impaired production of NADH is due to reduced activity of either PDHC or one or more steps in glycolysis. Impaired cerebral energy metabolism and PDHC activity are associated also with neurodegenerative disorders including Alzheimer's disease and Wernicke-Korsakoff syndrome, suggesting that this enzyme is an important link in the pathophysiology of both acute brain injury and chronic neurodegeneration. © 2004 Wiley-Liss, Inc.

**Key words:** mitochondria; peroxynitrite; acetyl-L-carnitine; lactate; acidosis

## PYRUVATE DEHYDROGENASE ENZYME COMPLEX

The pyruvate dehydrogenase complex (PDHC), located in the mitochondrial matrix, plays a major role in aerobic energy metabolism. This enzyme serves as the critical link between glycolysis (anaerobic metabolism) and the tricarboxylic acid cycle by catalyzing the oxidative

decarboxylation of pyruvate to form acetyl CoA (Fig. 1) (Reed, 1981, 2001). The PDHC is a multisubunit complex composed of three major subunits: E1, E2, and E3 (Fig. 1). The E1 subunit (pyruvate dehydrogenase) is a tetramer that contains two  $\alpha$  and two  $\beta$  subunits with molecular weights of 41 and 36 kDa, respectively. The entire PDHC contains approximately 30 copies of E1 and 60 copies of the 74-kDa E2 (dihydrolipoyl transacetylase) subunit. The 55-kDa E3 (dihydrolipoyl dehydrogenase) subunit is also found in  $\alpha$ -ketoglutarate dehydrogenase ( $\alpha$ -KGDH) (Patel and Harris, 1995). This shared homology makes many of the pathologies observed in PDHC also relevant to  $\alpha$ -KGDH. Six copies of E3 are found within the PDHC. The enzyme complex also requires a variety of substrates and cofactors. Pyruvate, nicotinamide adenine dinucleotide (NAD<sup>+</sup>), thiamine pyrophosphate (TPP), and coenzyme A (CoA) are all required for PDHC activity, as are flavin adenine dinucleotide (FAD) and lipoic acid (Reed, 2001). Activity of this complex enzyme is regulated by a host of factors, including phosphorylation state, Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]), [Mg<sup>2+</sup>] (Huang et al., 1998), and [ATP/ADP] ratio (Fig. 2). PDHC is phosphorylated and therefore inactivated by PDH kinase, whereas PDH phosphatase activates the enzyme complex. Four isozymes of mammalian PDH kinase have been identified (PDK1–4). Although these isozymes differ in tissue-specific expression (Bowker-Kinley et al., 1998), they are all activated by elevated [acetyl-CoA/CoA] and [NADH/NAD<sup>+</sup>] ratios (Bowker-Kinley, et al., 1998; Baker et al., 2000; Sugden and Holness, 2003) and inhibited by elevated ADP levels (Roche et al., 2003) and the drug

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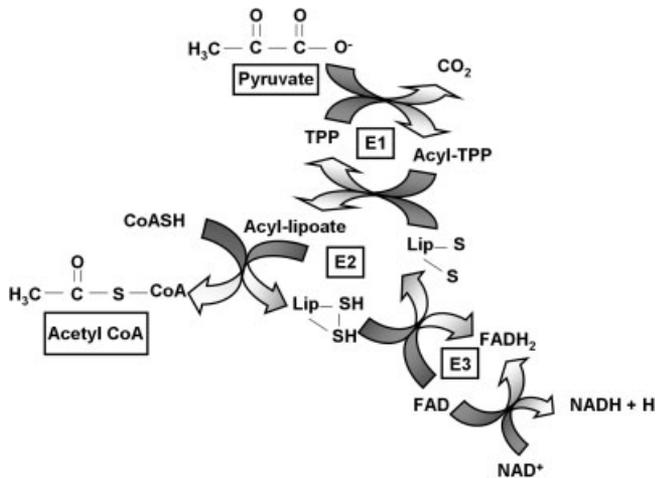


Fig. 1. Partial reactions of the PDHC. The PDHC catalyzes the oxidative decarboxylation of pyruvate to form acetyl CoA. The enzyme complex is composed of three major subunits: pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). In addition to pyruvate and coenzyme A (CoASH), PDHC also requires a host of cofactors, including thiamine pyrophosphate (TPP), nicotinamide adenine dinucleotide (NAD<sup>+</sup>), flavin adenine dinucleotide (FAD), and lipoic acid (LIP).

dichloroacetate (Whitehouse et al., 1974; Baker et al., 2000; Wilson et al., 2003). Some PDH phosphatase isoforms are stimulated by Ca<sup>2+</sup> (Huang et al., 1998; Karpova et al., 2003; Roche et al., 2003). The complexity of the multitude of subunits, strict cofactor requirements, and stringent regulation of the PDHC make it a possible target for damage and subsequent inactivation during pathologic conditions including ischemia and neurodegenerative disorders.

**EFFECTS OF ISCHEMIA/REPERFUSION ON CEREBRAL ENERGY METABOLISM AND PDHC ACTIVITY**

Measurements of cerebral glucose metabolism and oxygen utilization after global ischemia/reperfusion indicate that cerebral energy metabolism is impaired markedly during postischemic recirculation (Pulsinelli et al., 1982). A significant decrease in glucose oxidation develops within the first hour of reperfusion and remains for many hours thereafter (Sims, 1995). Oxidative glucose metabolism is also reduced after focal cerebral ischemia in a time-dependent manner (Pascual et al., 1998). Although aerobic glucose metabolism is impaired, the oxidative metabolism of other fuels, e.g., glutamate,  $\gamma$ -aminobutyric acid (GABA), and glutamine, can accelerate after focal ischemia (Pascual et al., 1998).

One possible explanation for reduced cerebral glucose metabolism after ischemia is decreased PDHC activity (Fukuchi et al., 1998; Schoder et al., 1998). In the rat dorsolateral striatum after short-term forebrain ischemia, PDHC activity is reduced, particularly in selectively vulnerable neurons (Zaidan and Sims, 1997). Reduced

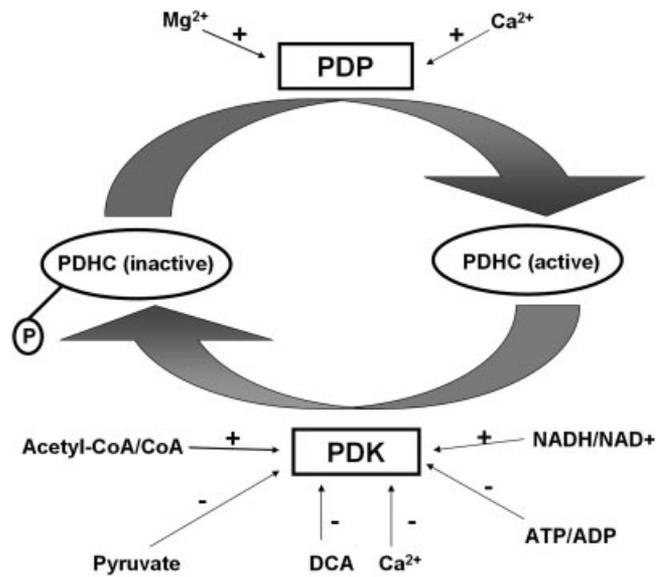


Fig. 2. Regulation of PDHC by phosphatase and kinase activities. PDHC is inactivated when phosphorylated by PDH kinase (PDK). PDK is activated by elevated NADH/NAD<sup>+</sup> and acetyl CoA/CoA ratios and is inactivated by pyruvate, dichloroacetate (DCA), Ca<sup>2+</sup>, and elevated ATP/ADP ratios. PDHC is activated when dephosphorylated by PDH phosphatase (PDP). Although less characterized than PDK, PDP is stimulated by both Mg<sup>2+</sup> and Ca<sup>2+</sup>. Four isozymes of PDH kinase (PDK1-4) and two isozymes of PDH phosphatase (PDP1c and PDP2c) have been identified. These isozymes differ in tissue specificity, as well as relative sensitivity to the effectors depicted in the figure.

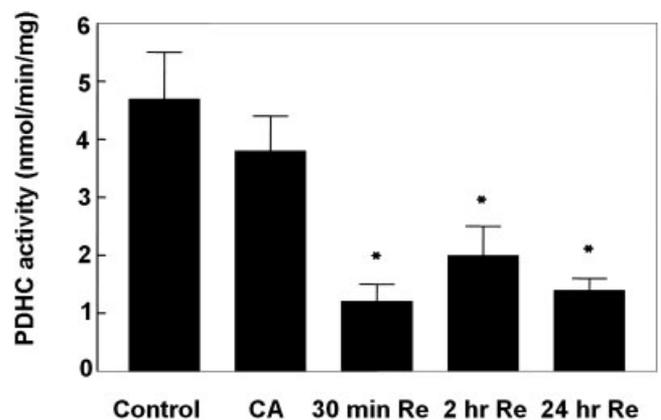


Fig. 3. Effects of cardiac arrest and resuscitation on canine frontal cortex PDHC enzyme activity. PDHC activity remains unchanged after 10 min of cardiac arrest (CA) alone. Activity is decreased significantly, however, as early as 30 min of reperfusion (Re) and remains depressed through 24 hr. PDHC activity was measured using a radioisotopic assay that monitors CO<sub>2</sub> production from [1-<sup>14</sup>C] pyruvate. PDHC activity is reported in nmol/min/mg total brain protein. Reprinted from Free Radical Biology and Medicine, Vol 16, Bogaert et al., Postischemic inhibition of cerebral cortex pyruvate dehydrogenase, p 811-820, ©1994 with permission from Elsevier.

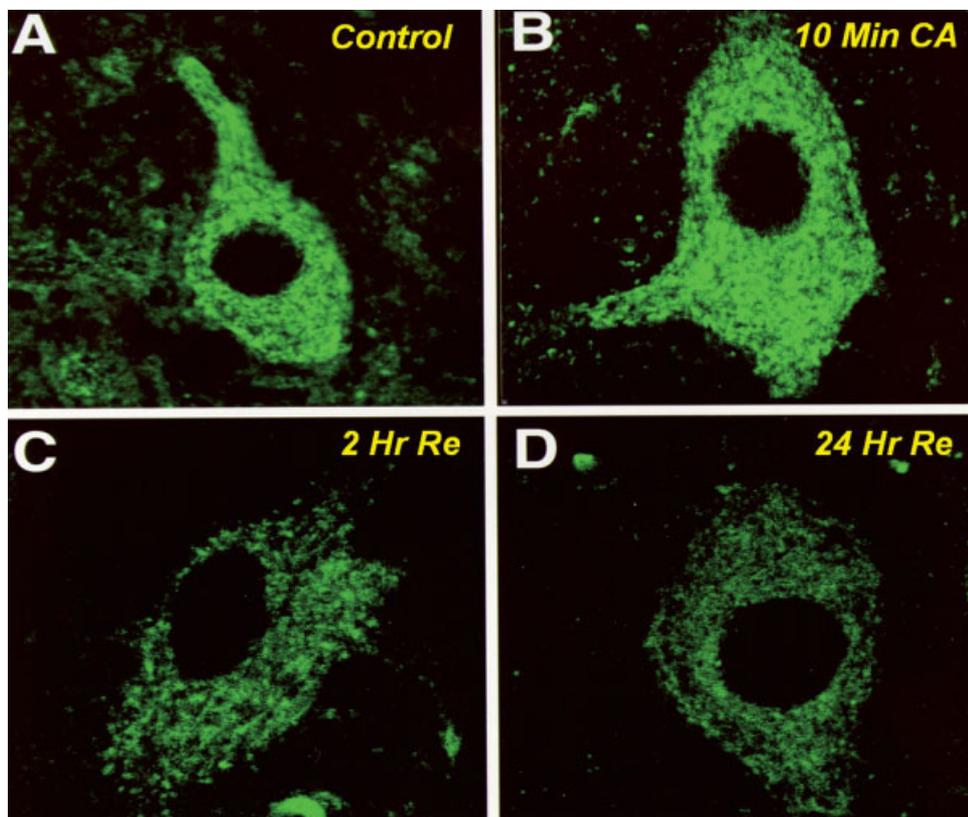


Fig. 4. Effects of cardiac arrest and resuscitation on canine frontal cortex PDHC immunoreactivity. Canine frontal cortex neuronal PDHC immunoreactivity remains unchanged after 10-min cardiac arrest (A, B). Confocal fluorescent imaging, using a polyclonal antibody to the entire PDHC complex, reveals diminished immunoreactivity after 2-hr reperfusion (C), which remains low at 24 hr reperfusion (D). Reprinted from *Experimental Neurology*, Vol 161, Bogaert et al., Neuronal subclass-selective loss of pyruvate dehydrogenase immunoreactivity following canine cardiac arrest and resuscitation, p 115-126, ©2000 with permission from Elsevier.

PDHC activity is observed in other locations, such as the frontal cortex, and is evident with reperfusion times as short as 30 min and as long as 24 hr (Zaidan and Sims, 1993; Bogaert et al., 1994). This decrease in enzyme activity is reperfusion dependent, as no change in activity is detected after ischemia alone (Fig. 3). It is unlikely that these changes are attributed to changes in cofactor levels, as the activity assays were carried out with saturating levels of required substrates and cofactors. PDHC immunoreactivity is also lost after ischemia/reperfusion. After a 10-min canine cardiac arrest with either 2 or 24 hr of reperfusion, both Western immunoblot and immunohistochemical analysis indicate a significant decrease in PDHC immunoreactivity (Fig. 4) (Bogaert et al., 2000). It is unlikely that the decline in PDHC activity or immunoreactivity is due to changes in the phosphorylation of the complex, as the phosphorylation state of PDHC does not change appreciably, at least in some models of ischemia/reperfusion (Zaidan and Sims, 1993). The decreased PDHC immunoreactivity, however, could be due to effects of ischemia/reperfusion-mediated damage to one or more of the PDHC required substrates or cofactors. A more likely explanation is site-specific protein oxidation, which may cause the decreased PDHC immunoreactivity by marking the affected regions for proteolytic degradation after an ischemia/reperfusion event (Stadtman, 1990).

At this juncture, direct evidence for a reduction in PDHC activity being responsible for impaired postisch-

emic cerebral energy metabolism is lacking; however, several observations support this hypothesis. One such finding is that immediate intravenous administration of acetyl-L-carnitine (ALCAR; 100 mg/kg) reduces brain lactate levels and improves neurologic outcome after cardiac arrest (Table I) (Rosenthal et al., 1992). These effects are not observed with administration of equimolar equivalent levels of acetate and free carnitine, indicating that ALCAR possesses unique neuroprotective characteristics. Medium- and long-chain acylcarnitines are not well metabolized in the adult brain due to relatively very low acylcarnitine-CoA transferase activities. Acetylcarnitine-CoA transferase is present, however, allowing for possible entry of ALCAR acetyl units into the tricarboxylic acid (TCA) cycle of astrocytes or neurons (Bresolin et al., 1982). By providing a source of fuel alternative to pyruvate, ALCAR may stimulate aerobic energy metabolism, thereby reducing the rate of glycolytic lactate production and the tissue acidosis that accompanies anaerobic metabolism (Fig. 5). ALCAR also acts at least indirectly as an antioxidant, reducing protein carbonyl formation during reperfusion (Liu et al., 1993), and cerebrospinal fluid protein nitration in multiple sclerosis patients (Calabrese et al., 2003). This protection against oxidative stress may explain its ability to protect against the loss of PDHC activity in the cardiac arrest model (Bogaert et al., 1994), which in turn may help explain its ability to lower tissue lactate levels. ALCAR can also ameliorate some metabolic ab-

**TABLE I. Effects of Acetyl-L-Carnitine and Acetate Plus Carnitine on 2-hr Neurochemical Outcome and 24-hr Neurologic Outcome After 10-min Canine Cardiac Arrest**

	Vehicle <sup>a</sup>	Acetyl-L-carnitine <sup>a</sup>	Acetate + carnitine <sup>b</sup>
Lactate ( $\mu\text{mol/g}$ wet wt)	4.3 $\pm$ 0.2	2.0 $\pm$ 0.4*	5.7 $\pm$ 2.2
Pyruvate (nmol/g wet wt)	160 $\pm$ 41	197 $\pm$ 24	139.2 $\pm$ 51
Lactate/pyruvate	34.3 $\pm$ 6.9	9.5 $\pm$ 1.1**	51.3 $\pm$ 22
Neurodeficit score <sup>c</sup>	48.4 $\pm$ 5.4	22.3 $\pm$ 5.2***	41.0 $\pm$ 3.1

<sup>a</sup>Values for vehicle- and acetyl-L-carnitine-treated animals reprinted with permission from Rosenthal et al. 1992. Prevention of postischemic canine neurological injury through potentiation of brain energy metabolism by acetyl-L-carnitine. *Stroke* 23:1312-1318.

<sup>b</sup>Animals treated with acetate plus carnitine at levels equimolar to that of acetyl-L-carnitine administered at 100 mg/kg intravenously immediately after resuscitation, then at 50 mg/kg every 6 hr. Samples were obtained from canine frontal cortex, immediately placed into liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until analyzed for lactate and pyruvate. Values represent means  $\pm$  standard error for  $n = 5-7$  animals per group.

<sup>c</sup>Neurodeficit score evaluated on a scale from 0 (normal) to 100 (braindead).

\* $P < 0.01$  compared to vehicle group.

\*\* $P < 0.05$  compared to vehicle group.

\*\*\* $P < 0.002$  compared to vehicle group.

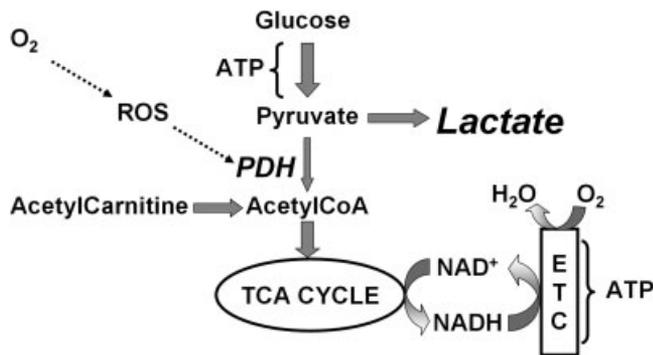


Fig. 5. PDHC serves as the bridge between anaerobic and aerobic metabolism. The PDHC is a target of oxidative stress and is inhibited after cerebral ischemia. Such inhibition may be responsible for chronically elevated brain lactate levels after ischemic episodes as PDHC constitutes the bridge between aerobic and anaerobic cerebral energy metabolism. Acetyl-L-carnitine (ALCAR) may serve as an exogenous, alternative source of acetyl CoA, thereby reducing tissue acidosis and improving neurologic outcome.

normalities induced by chronic excessive alcohol consumption, which is also associated with impaired brain PDHC activity (Calabrese et al., 2002).

Another observation implicating the role of PDHC in brain injury resulting from ischemia/reperfusion is the hyperoxidation of NAD(H) and components of the mitochondrial electron transport chain during reperfusion (Rosenthal et al., 1995). If damage to the electron transport chain was the major factor in ischemia/reperfusion injury, the redox state of NAD(H) would undergo a shift toward a more reduced state. The limiting factor in reperfusion injury therefore seems to be proximal to the electron transport chain. The rate-limiting site could include various TCA cycle enzymes, e.g.,  $\alpha$ -KGDH. According to the direct metabolic hypothesis for neuroprotection by ALCAR, however, inhibition of metabolism at points

distal to acetyl CoA would not be alleviated by ALCAR administration. Experiments are in progress using  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy to study the metabolism of ALCAR, and the effects it has on glucose metabolism to help determine the significance of altered PDHC activity in ischemic brain injury.

#### ROLE OF PYRUVATE DEHYDROGENASE COMPLEX IN THE PATHOPHYSIOLOGY OF NEURODEGENERATIVE DISORDERS

In addition to ischemic brain injury, PDHC is affected also in other neurologic disorders. Wernicke-Korsakoff Syndrome (WKS) is a disease characterized by a triad of mental confusion, ataxia, and ophthalmoplegia. This disorder is commonly associated with chronic alcoholism, although other nutritional deficits resulting in low dietary thiamine can also cause onset of disease symptoms. The main etiologic factor is known to be lack of thiamine, but the biochemical mechanisms involved remain unclear. Thiamine-dependent enzymes such as PDHC and  $\alpha$ KGDH are thought to play a role in the pathogenesis of WKS. Decreased PDHC and  $\alpha$ KGDH enzyme activities are found in alcoholics diagnosed with WKS, whereas alcoholic patients without WKS display normal levels of thiamine-dependent enzyme activities (Butterworth et al., 1993). Although decreased  $\alpha$ KGDH activity has also been shown in an animal model of thiamine deficiency, the effects of thiamine depletion on PDHC activity in vitro remain controversial (Parker et al., 1984; Butterworth and Heroux, 1989; Munujos et al., 1996). PDHC activity is also affected in Alzheimer's disease (AD) (Sheu et al., 1985). Brain lipid peroxidation and decreased brain glucose utilization are characteristic of this neurodegenerative disease. Acrolein, a byproduct of lipid peroxidation that accumulates within the brain during AD, decreases PDHC activity. Specifically, acrolein binds lipoic acid, a component of both PDHC and  $\alpha$ KGDH (Pocernich and Butterfield, 2003). Inactivation of PDHC by acrolein or other mechanisms may be at least partially responsible for mito-

chondrial dysfunction and impaired cerebral energy metabolism associated with AD.

### MOLECULAR MECHANISMS OF ENZYME INACTIVATION

The PDHC inactivation that occurs during acute brain injuries and in neurodegenerative disorders could be due to any one or a combination of several mechanisms. In addition to depletion of the enzyme cofactors TPP and lipoic acid, the protein subunits may also be direct targets of oxidative stress. Purified porcine heart PDHC is highly sensitive to inactivation when exposed to a hydroxyl radical ( $\text{OH}^\bullet$ ) generating system composed of  $\text{H}_2\text{O}_2$  and  $\text{Fe}^{2+}$  (Bogaert et al., 1994). PDHC activity is not lost in the presence of  $\text{H}_2\text{O}_2$  alone, as activity is retained in the presence of the iron chelator diethylenetriaminepentaacetic acid (DTPA; 2 mM) (Fig. 6). Recent findings in our laboratory suggest that PDHC is also targeted by peroxynitrite ( $\text{ONOO}^-$ ). Peroxynitrite is formed when superoxide reacts with NO (Beckman et al., 1990; Goldstein and Czapski, 1995; Beckman, 1996; Murphy et al., 1998). Both substrates are generated by 3-morpholininosynonimine (SIN-1) (Feelisch et al., 1989), which when incubated with purified PDHC results in a loss of enzyme activity that is partially inhibited by the presence of superoxide dismutase (Fig. 6). Further experiments are planned to determine the relative contribution of  $\text{ONOO}^-$ , compared to NO or  $\text{O}_2^{\bullet-}$ , to the impairment of enzyme activity observed in this system.

In addition to the aforementioned *in vitro* effects of free radicals on PDHC activity, decreased activity of other mitochondrial proteins has also been identified *in vivo*. Hypoxia-reoxygenation paradigms decrease aconitase and succinate dehydrogenase activities, a finding attributed to excess  $\text{O}_2^{\bullet-}$  production (Powell and Jackson, 2003). Additionally,  $\alpha\text{KGDH}$  and aconitase activities are decreased during reperfusion of ischemic myocardial tissue (Sadek et al., 2002). As evidence indicates that production of superoxide, hydroxyl radical, nitric oxide, and peroxynitrite are elevated during reperfusion (Metodiewa and Koska, 2000), these results support the hypothesis that oxidative stress is responsible for reperfusion-dependent loss of brain PDHC activity.

Further support for oxidative stress as a mechanism responsible for PDHC damage during ischemia reperfusion comes from comparison of protein immunoreactivity between cardiac arrest animal groups resuscitated with relatively high and low concentrations of ventilatory  $\text{O}_2$ . Hippocampal PDHC E1 $\alpha$  subunit immunostaining is reduced by up to 90% within 2 hr of reperfusion in dogs resuscitated on 100%  $\text{O}_2$  compared to that in nonischemic animals, whereas no significant reduction in hippocampal E1 $\alpha$  immunoreactivity is observed in animals resuscitated with 21%  $\text{O}_2$  (room air). Double labeling with neuron-specific nuclear protein antibody (NeuN) indicates the PDHC loss is partially neuronal, but astrocytic involvement remains undetermined. Moreover, hippocampal nitrotyrosine immunoreactivity is greater in the hyperoxic resuscitation group (Vereczki et al., 2003). These results,

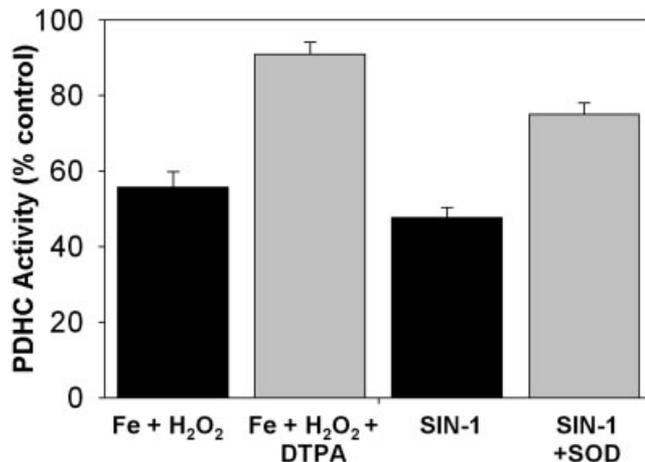


Fig. 6. Inhibition of PDHC activity by hydroxyl radical and peroxynitrite. Results obtained with exposure of purified porcine heart mitochondria for 10 min at 37°C to a Fenton reagent consisting of 0.25 mM  $\text{FeSO}_4$  plus 0.5 mM  $\text{H}_2\text{O}_2$  in the absence and presence of 2.5 mM diethylenetriaminepentaacetic acid (DTPA). Reprinted from *Free Radical Biology and Medicine*, Vol 16, Bogaert et al., Postischemic inhibition of cerebral cortex pyruvate dehydrogenase, p 811–820, ©1994 with permission from Elsevier. Additional results were obtained under similar conditions but using 0.6 mM of the peroxynitrite generator 3-morpholininosynonimine (SIN-1) in the absence and presence of superoxide dismutase (SOD; 20 U/ml). Enzyme activity after the preincubations was measured either radioisotopically by determining the  $^{14}\text{CO}_2$  production from [1- $^{14}\text{C}$ ] pyruvate (Fenton reagent; Bogaert et al., 1994), or spectrophotometrically by measuring NADH production at 340 nm (SIN-1). Dithiothreitol (9 mM) was present during all preincubations to eliminate possible inactivation due to thiol oxidations or S-nitrosylation. Experimental conditions for experiments involving SIN-1 were modified from Hinman and Blass (1981) and consisted of 50 mM potassium phosphate buffer (37°C), 2.06 U/ml PDHC, 5 mM pyruvate, 0.3 mM thiamine pyrophosphate (TPP), 1 mM magnesium chloride, 0.01 mM calcium chloride, and 1 mM  $\text{NAD}^+$ . The reaction was initiated by the addition of 0.12 mM coenzyme A whereas the reactions after exposure to the Fenton reagent were initiated with the addition of 2 mM pyruvate.

taken together with our previous findings that hyperoxic resuscitation causes increased brain lipid peroxidation and worse neurologic outcome (Liu et al., 1998), suggest that PDHC is one important target of oxidative stress and that its inactivation may contribute to neuronal injury and neurologic impairment.

### POTENTIAL THERAPEUTIC INTERVENTIONS

Based on the hypothesis that impairment of PDHC activity contributes to the pathophysiology of ischemic brain injury, interventions that either protect against PDHC inactivation or compensate for the metabolic disruption should be neuroprotective. Dichloroacetate (DCA) is a pharmacologic agent that stimulates maximal PDHC activity by inhibiting PDH kinase. It is used as a treatment for patients with PDHC deficiency, a condition that presents clinical symptoms during the first months of

life. PDHC activity is increased in PDHC-deficient patients treated with 5 mM DCA (Fouque et al., 2003). DCA administration has also been demonstrated to decrease brain lactate and to improve outcome in small and large animal models of both global and focal cerebral ischemia (Biros et al., 1986; Cardell et al., 1989; Katayama and Welsh, 1989; Chang et al., 1992; Corbett et al., 1998; Chandy and Ravindra, 2000). Moreover, one clinical study using proton magnetic resonance spectroscopy indicates that administration of DCA within the first 2 days of ischemic stroke lowers brain lactate (Graham et al., 2000). Administration of lipoic acid is also neuroprotective and although its mechanisms of action are ascribed to either direct antioxidant activity or regulation of gene transcription, promotion of PDHC activity has not been addressed (Wolz and Krieglstein, 1996; Packer, 1998; Clark et al., 2001; Garcia-Estrada et al., 2003). Thiamine replacement therapy is used for patients with thiamine deficiency but has not been tested for neuroprotection after acute brain injury.

As mentioned earlier, immediate postischemic infusion of acetyl-L-carnitine is neuroprotective, possibly by providing alternative oxidative fuel in the presence of reduced conversion of pyruvate to acetyl CoA. Other researchers have demonstrated neuroprotection by administration of ketone bodies, e.g.,  $\beta$ -hydroxybutyrate, in animal models of ischemia, trauma, and Parkinson's disease (Lundy et al., 1984, 1987; Marie et al., 1987; Sims and Heward, 1994; Kashiwaya et al., 2000; Ottani et al., 2003; Tieu et al., 2003; Yosunkaya et al., 2004). Although the mechanism of neuroprotection by ketone bodies is not characterized, they similarly bypass the PDHC reaction and provide fuel to the TCA cycle.

Although interventions that compensate for a loss of PDHC activity may prove clinically effective, an alternative approach is to inhibit those mechanisms responsible for damage to the enzyme complex. Based on the hypothesis that oxidative stress is the primary culprit, antioxidants should prove useful. Indeed, many preclinical studies with antioxidants have demonstrated neuroprotection (Chan, 2001; Floyd and Hensley, 2002); however, results from the few clinical trials testing antioxidants are disappointing (van der Worp et al., 2002). An alternative approach to minimizing oxidative stress during postischemic reperfusion is to limit the delivery of oxygen to the brain. The few reported comparisons of neurologic outcome after hyperoxic and normoxic reperfusion strongly suggest that hyperoxic resuscitation is detrimental. Using a 9-min canine cardiac arrest (CA) model, Zwemer et al. (1994) found that resuscitation with 100% inspired  $O_2$  resulted in worsened 12- and 24-hr neurologic outcome when compared to that in animals receiving 21%  $O_2$ . This difference was eliminated when animals were pretreated with an antioxidant before the CA and hyperoxic resuscitation. In our canine experiments using 10-min CA, neurologic impairment measured at 24 hr was significantly worse in animals ventilated on 100%  $O_2$  during and for 1 hr after resuscitation than that exhibited by dogs resuscitated on

21%  $O_2$  and subsequently ventilated on 21–30%  $O_2$  to maintain normal  $PaO_2$  (Rosenthal et al., 2003). The one negative study is the report mentioned earlier where no difference in neurologic impairment was observed 72 hr after asphyxia-induced CA in rats (Lipinski et al., 1999). The only published long-term outcome study focused on mortality and used the gerbil bilateral carotid occlusion model. Mickel et al. (1987) found that animals exposed to 100%  $O_2$  for 3–6 hr after 15-min global cerebral ischemia experienced a threefold increase in 14-day mortality compared to those allowed to breathe room air after ischemia. As mentioned earlier, our recent results comparing hippocampal PDHC immunoreactivity between hyperoxic and normoxic resuscitated animals indicate that normoxic ventilation preserves this PDHC immunostaining (Vereczki et al., 2003). Studies are in progress to determine if normoxic resuscitation also preserves PDHC enzyme activity and, in turn, aerobic cerebral energy metabolism.

### SUMMARY

PDHC plays a critical role in cerebral aerobic energy metabolism. Its vast size, strict cofactor requirements, and stringent regulation make it a potential target of injury during times of neurologic stress, such as ischemia or trauma. Indirect evidence suggests that loss of PDHC enzyme activity after cardiac arrest and resuscitation contributes to the prolonged elevation of brain lactate levels. More direct evidence that impaired PDHC activity limits cerebral energy metabolism during reperfusion awaits quantitative analysis of metabolic flux. Although evidence obtained *in vitro* indicates that PDHC is sensitive to inactivation when exposed to reactive oxygen and nitrogen species, further work is necessary to conclude that oxidative alterations are responsible for its inactivation *in vivo*.

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