Oxidative stress reversibly inactivates myocardial enzymes during cardiac arrest

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Oxidative stress reversibly inactivates myocardial enzymes during cardiac arrest. Am J Physiol Heart Circ Physiol 292: H198–H206, 2007. First published August 18, 2006; doi:10.1152/ajpheart.00698.2006.—Oxidative stress during cardiac arrest may inactivate myocardial enzymes and thereby exacerbate ischemic derangements of myocardial metabolism. This study examined the impact of cardiac arrest on left ventricular enzymes. Beagles were subjected to 5 min of cardiac arrest and 5 min of open-chest cardiac compressions (OCCC) before epidural direct current countershocks were applied to restore sinus rhythm. Glutathione/glutathione disulfide redox state (GSH/GSSG) and a panel of enzyme activities were measured in snap-frozen left ventricle. To test whether oxidative stress during arrest inactivated the enzymes, metabolic (pyruvate) or pharmacological (N-acetyl-L-cysteine) antioxidants were infused intravenously for 30 min before arrest. During cardiac arrest, activities of phosphofructokinase, citrate synthase, aconitase, malate dehydrogenase, creatine kinase, glucose-6-phosphate dehydrogenase, and glutathione reductase fell by 56, 81, 55, 34, 42, 55, and 45%, respectively, coincident with 50% decline in GSH/GSSG. OCCC effected full recovery of glutathione reductase and partial recovery of citrate synthase and aconitase, in parallel with GSH/GSSG. Phosphofructokinase, malate dehydrogenase, creatine kinase, and glucose-6-phosphate dehydrogenase recovered only after cardioversion. Antioxidant pretreatments augmented phosphofructokinase, aconitase, and malate dehydrogenase activities before arrest and enhanced these activities, as well as those of citrate synthase and glucose-6-phosphate dehydrogenase, during arrest. In conclusion, cardiac arrest reversibly inactivates several important myocardial metabolic enzymes. Antioxidant protection of these enzymes implicates oxidative stress as a principal mechanism of enzyme inactivation during arrest.

glutathione; pyruvate; N-acetyl-L-cysteine; phosphofructokinase; creatine kinase; citrate synthase

CARDIAC ARREST IMPOSES severe myocardial ischemia that depletes high-energy phosphates. Reactive oxygen species (ROS) generated during ischemia (4, 31) can modify cellular components, including phospholipids, structural proteins, ion transporters, and enzymes. Inactivation of metabolic enzymes can exacerbate ischemic derangement of myocardial energy and antioxidant reserves.

Several myocardial enzymes are susceptible to modification and inactivation by ROS, including enzymes of glycolysis [phosphofructokinase (13, 21, 24) and glyceraldehyde-3-phosphate dehydrogenase (22)], tricarboxylic acid (TCA) cycle [aconitase (10, 14) and malate dehydrogenase (17)], intracellular energy transport [creatinine kinase (24, 40)], and antioxidant defense [glutathione reductase (41), glutathione peroxidase(41), and glucose-6-phosphate dehydrogenase (26)]. Enzyme modifications may be reversible or permanent, depending on the severity of oxidative stress, culprit oxidant species, and chemical nature of the modification. Cells are armed with enzyme systems that can reverse some protein modifications and restore protein function. These systems include the protein disulfide reductases thioredoxase and thioredoxin (2), glutaredoxins (29) catalyzing glutathionedisulfide oxidoreductions, and sulfiredoxin (7), which reduces cysteine sulfenic acid derivatives formed by oxidation of cysteine residues. Other modifications, such as carbonylation, are irreversible and divert proteins to ubiquitin and proteosomal degradation (16, 31).

This study examined the impact of cardiac arrest and resuscitation on a panel of metabolic enzymes in left ventricular myocardium. Beagles underwent 5 min of cardiac arrest, followed by 5 min of open-chest cardiac compressions (OCCC), defibrillation, and 3 h of recovery. Myocardial enzyme activities were measured in left ventricular biopsies. Several enzymes were partially inactivated during cardiac arrest and recovered after sinus rhythm was restored. The changes in enzyme activities paralleled glutathione redox state, a measure of myocardial antioxidant state. To test whether oxidative stress inactivated the enzymes, antioxidants pyruvate and N-acetyl-L-cysteine (NAC) were administered before arrest. The antioxidant pretreatments prevented subsequent collapse of several enzyme activities during cardiac arrest, in some cases by increasing prearrest activities.

METHODS

All animal experimentation conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, Revised 1996) and was approved by the Institutional Animal Care and Use Committee of the University of North Texas Health Science Center. Adult beagles (11.3 ± 0.3 kg; 40 males, 51 females) were randomly assigned to either cardiac arrest-resuscitation or the antioxidant pretreatment groups described below.

Surgical preparation and instrumentation. After an overnight fast, dogs were sedated with morphine sulfate (3 mg/kg sc) and anesthetized with α-chloralose (100 mg/kg iv). Supplemental α-chloralose was administered as needed to maintain anesthesia. Surgical preparation and instrumentation were performed as recently described (35). Briefly, the dogs were intubated and mechanically ventilated with room air enriched with supplemental O2. Vinyl catheters were placed in the abdominal aorta via femoral artery for sampling arterial blood and measuring blood pressure, in a femoral vein for antioxidant infusion, and in the other femoral vein for administration of supplemental anesthetic and NaHCO3. Arterial 

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pH, PO₂, and PCO₂ were maintained within physiological limits by adjusting tidal volume and ventilatory frequency, ventilating with supplemental O₂ and administering NaHCO₃. Body temperature was maintained at 36–37°C by use of a circulating water heating pad.

The heart was exposed via left lateral thoracotomy in the fifth intercostal space and suspended in a pericardial cradle. A vinyl cannula was placed in the right atrium to administer intracardiac medications. Lead II electrocardiography was used to confirm ventricular fibrillation during arrest and sinus rhythm after cardiovascular return.

Cardiac arrest and cardiopulmonary resuscitation protocol. Cardiopulmonary arrest (35) was induced by applying a 9-V current to the left ventricular epicardium and interrupting mechanical ventilation. At 4.5 min of arrest, a 1-mg epinephrine bolus was injected into the right atrium. OCCC (80 compressions/min) were administered at 5–10 min of cardiac arrest to achieve a mean aortic pressure of ~60 mmHg. Mechanical ventilation was resumed at 12 cycles/min during OCCC. Beginning at 10 min of arrest, defibrillatory direct current countershocks were delivered to the epicardium with internal paddles. Up to four 5-J countershocks with intervening OCCC were delivered at 30-s intervals, followed by up to three 10-J countershocks, until cardiovascular return was achieved.

Recovery of spontaneous circulation (ROSC) was defined as mean aortic pressure of ≥60 mmHg and spontaneous cardiac rhythm. In the event of ventricular tachyarrhythmia, lidocaine (6 mg bolus) was injected into the right atrium to restore sinus rhythm. Experiments were terminated by collecting freeze-clamp biopsies of the left ventricular anterior wall at prearrest baseline, 5 min of arrest, 5 min of OCCC, 25 min of ROSC, and 180 min of ROSC, using liquid N₂-precooled Wollenberger tongs. Biopsies were stored at ~90°C before analyses of metabolites and enzymes.

Antioxidant pretreatments. Following surgical instrumentation and stabilization of cardiac function, the dogs received intravenous infusion of either sodium pyruvate (0.125 mmol/kg) and stabilization of cardiac function, the dogs received intravenous infusion of either sodium pyruvate (0.125 mmol/kg) and NAC (0.06 mmol/kg) or NAC (0.06 mmol/kg), a pharmacological antioxidant (27). At 30 min of infusion, the heart was arrested and antioxidant infusion was simultaneously discontinued. Left ventricular myocardium was biopsied at 30 min of prearrest infusion or at 5 min of arrest.

Analytical procedures. Myocardial metabolites were extracted and measured by spectrophotometric assays (24). Arterial plasma was obtained by centrifugation of freshly drawn whole blood and flash frozen in liquid N₂. Glutathione/glutathione disulfide redox state (GSH/GSSG) and NAC concentration in plasma were measured by high-performance liquid chromatography with fluorescence detection (24). 8-Isoprostane, a product of nonenzymatic oxidative modification of tissue phospholipids, is considered to be a reliable marker of oxidative stress (19). Competitive enzyme immunoassay for 8-isoprostane (Cayman Chemical, Ann Arbor, MI) was performed in arterial plasma, according to the manufacturer’s instructions.

Enzymes were extracted from snap-frozen left ventricular myocardium (24). Briefly, tissue was pulverized to a fine powder in a precooled mortar under liquid N₂, then homogenized in 6–8 vol ice-cold buffer solution. Sulfhydryl reducing agents were omitted from the buffer to avoid spontaneous recovery of enzymes inactivated by cardiac arrest. Following centrifugation (20 min at 4°C and 116,000 gmax), the pellet was reextracted twice in 4 vol phosphate buffer, and the three supernatant fractions were combined and stored at ~90°C. Enzyme activities were measured at 37°C in a Shimadzu Instruments model UV-1601 dual wavelength UV-vis spectrophotometer according to spectrophotometric methods described in Bergmeyer (6). Activities were determined from the rates of formation or disappearance of NADPH or NADH monitored at 337-nm wavelength (ε = 6.24 mM⁻¹cm⁻¹). Extract protein concentration was measured with the Coomassie plus kit (Pierce, Rockford, IL). Enzyme activities were expressed as units per milligram protein, where 1 U equals 1 μmol substrate converted to product per minute. To exclude the possibility that antioxidant enhancements of enzyme activities resulted from residual antioxidant in the snap-frozen myocardium, untreated myocardium was extracted in the absence and presence of added pyruvate and NAC (0.8 μmol/g).

Statistical analyses. Data are reported as means ± SE. Between-group comparisons of myocardial metabolites, GSH/GSSG, and enzyme activities were accomplished by single-factor ANOVA. Single-factor ANOVA also was used for within-group comparisons of myocardial enzymes and metabolites at different time points. Repeated-measures ANOVA was used for within-group comparisons of enzymatic activities during cardiac arrest, open-chest cardiac compressions (OCCC), and recovery of spontaneous circulation (ROSC). Activities of phosphofructokinase (PFK, A), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, B), and lactate dehydrogenase (LDH, C) were measured at prearrest baseline (BL), 5 min of cardiac arrest (ARR), 5 min of OCCC, and at 25 and 180 min after ROSC. Solid bar, nonarrested sham-operated time control. Values are means ± SE from 6–9 experiments at each time point. *P < 0.05 vs. BL.

Fig. 1. Myocardial glycolytic enzyme activities during cardiac arrest, open-chest cardiac compressions (OCCC), and recovery of spontaneous circulation (ROSC). Activities of phosphofructokinase (PFK, A), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, B), and lactate dehydrogenase (LDH, C) were measured at prearrest baseline (BL), 5 min of cardiac arrest (ARR), 5 min of OCCC, and at 25 and 180 min after ROSC. Solid bar, nonarrested sham-operated time control. Values are means ± SE from 6–9 experiments at each time point. *P < 0.05 vs. BL.
comparisons of plasma GSH/GSSG, 8-isoprostane, and antioxidant concentrations. When ANOVA detected statistical significance, Student-Newman-Keuls multiple comparison test was applied to identify specific differences among mean values. *P values < 0.05 were taken to indicate statistically significant differences. Statistical analyses were performed by using SigmaStat version 3.1 software.

RESULTS

Enzyme inactivation during cardiac arrest and recovery during resuscitation and ROSC. The impact of cardiac arrest, cardiopulmonary resuscitation effected by OCCC, and ROSC on a panel of key metabolic enzymes was examined in canine left ventricular myocardium. Activity of phosphofructokinase, the principal rate-controlling glycolytic enzyme, fell by 56% during cardiac arrest, remained depressed during OCCC, and then slowly recovered during 3 h of ROSC (Fig. 1A). Two other glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase (Fig. 1B) and lactate dehydrogenase (Fig. 1C), were not appreciably inactivated during the protocol, although a trend toward lower lactate dehydrogenase activity was detected during arrest, OCCC, and at 25 min of ROSC. Among TCA cycle enzymes, citrate synthase activity fell the most steeply during cardiac arrest, by 81%, then substantially recovered during OCCC, and at 25 min of ROSC. Aconitase activity fell 55% during arrest and then gradually recovered during OCCC and ROSC (Fig. 2A). Aconitase activity fell 55% during arrest and then gradually recovered during OCCC and ROSC (Fig. 2B). Malate dehydrogenase activity fell 34% during arrest, remained depressed during OCCC, and recovered only after ROSC (Fig. 2C). Creatine kinase, which catalyzes energy transfer between mitochondria and extramitochondrial ATPases, fell 42% during arrest and did not return to sham-operated control level until 180 min of ROSC (Fig. 2D).

Cardiac arrest and resuscitation also affected enzyme components of the glutathione antioxidant system. Activities of glucose-6-phosphate dehydrogenase, a major source of NADPH, and glutathione reductase, which uses NADPH-reducing power to regenerate glutathione from glutathione disulfide, fell by 55% and 45%, respectively, during cardiac arrest (Fig. 3). Glutathione reductase quickly recovered during OCCC, but glucose-6-phosphate dehydrogenase only recovered after ROSC. Glutathione peroxidase harnesses glutathione-reducing power to detoxify H$_2$O$_2$ and lipid peroxides. Although cardiac arrest tended to inactivate glutathione peroxidase, the activity of the enzyme sharply increased to twice its baseline value during OCCC. Collectively, these results indicate that cardiac arrest reversibly inactivated several important metabolic enzymes. The enzymes spontaneously recovered, albeit at different rates, during OCCC and ROSC.

Glutathione redox state and arterial 8-isoprostane. Arterial plasma 8-isoprostane, a product of free radical oxidation of arachidonic acid and an index of systemic oxidative stress, increased sharply during OCCC and the first 5 min of ROSC and then gradually subsided (Fig. 4A). GSH/GSSG, a measure of myocardial antioxidant reducing power, fell sharply during arrest, then progressively recovered during OCCC and the first 25 min of ROSC (Fig. 4B).

Antioxidant pretreatments and glutathione redox state. To test the hypothesis that oxidative stress imposed by cardiac arrest inactivated myocardial enzymes, the ability of antioxidant pretreatments to protect enzyme activities during cardiac arrest was examined. The pharmacological antioxidant NAC or the natural metabolic antioxidant pyruvate was systemically infused for 30 min immediately before cardiac arrest. Arterial plasma pyruvate concentration sharply increased during intravenous pyruvate infusion and, within 8 min, stabilized at ~4 mM (Fig. 5A). Plasma NAC steadily increased over 30 min of infusion to a concentration of 1 ± 0.2 mM (Fig. 5B). NAC was...
undetectable in plasma of pyruvate-treated and sham-operated control dogs, and NAC infusion did not alter plasma pyruvate concentration.

NAC pretreatment enhanced glutathione redox state in both plasma (Fig. 6A) and myocardium (Fig. 6B). Pyruvate pretreatment modestly increased GSH/GSSG in plasma but not in myocardium. At 5 min of arrest, GSH/GSSG in NAC- or pyruvate-pretreated myocardium was 48% and 57% greater, respectively, than that of untreated myocardium (Fig. 6B). Thus antioxidant pretreatments partially preserved myocardial GSH reducing power during cardiac arrest.

Antioxidants protect myocardial enzymes during cardiac arrest. Antioxidants enhanced prerest activities of the glycolytic enzymes phosphofructokinase and lactate dehydrogenase (Fig. 7, A and B). During arrest, pyruvate pretreatment enhanced phosphofructokinase activity (Fig. 7A), and NAC pretreatment increased lactate dehydrogenase activity (Fig. 7B). Glyceraldehyde-3-phosphate dehydrogenase was unaltered by cardiac arrest or by antioxidants (Fig. 7C).

Neither pyruvate nor NAC affected citrate synthase activity before arrest, but both pretreatments, especially NAC, attenuated inactivation of this TCA cycle enzyme during cardiac arrest (Fig. 8A). Pyruvate but not NAC augmented aconitase activity before arrest, but only NAC pretreatment protected the enzyme during arrest (Fig. 8B). Both pretreatments increased malate dehydrogenase activity before and during cardiac arrest (Fig. 8C). Creatine kinase was not affected by antioxidant pretreatments (Fig. 8D), and antioxidant enhancements of this enzyme during arrest were not significant. Neither glucose-6-phosphate dehydrogenase, the rate-controlling enzyme of the hexose monophosphate shunt, nor glutathione reductase was affected by antioxidants before cardiac arrest, but NAC pretreatment protected both enzymes during arrest (Fig. 9, A and B). Both antioxidants markedly increased glutathione peroxidase before arrest, but only NAC pretreatment enhanced the activity of the enzyme during arrest (Fig. 9C).

Enzyme activities are not increased by residual antioxidants in myocardial homogenate. Residual NAC or pyruvate in homogenates of antioxidant-treated myocardium could conceivably protect enzymes from inactivation during extraction,

Fig. 3. Myocardial antioxidant enzymes. Activities of glucose-6-phosphate dehydrogenase (G6PDH, A), glutathione reductase (GR, B), and glutathione peroxidase (GPx, C) were measured at the same times and in the same experiments as in Figs. 1 and 2. *P < 0.05 vs. BL; †P < 0.05 vs. ARR.

Fig. 4. Measures of oxidative stress: plasma 8-isoprostane and myocardial glutathione redox state (GSH/GSSG). A: 8-isoprostane was measured in arterial blood samples at prerest BL, 3 min of ARR, 3 min of OCCC, and at several times following ROSC. *P < 0.05 vs. BL; †P < 0.05 vs. sham at same time. B: GSH/GSSG was measured in left ventricular myocardium at prerest BL, 5 min of ARR, 5 min of OCCC, and at 25 and 180 min after ROSC. Solid bar, nonarrested sham-operated time control. Values are means ± SE from the same experiments as in Figs. 1–3. *P < 0.05 vs. BL; †P < 0.05 vs. ARR.
which would artifactually increase enzyme activities. To test this possibility, 0.8 μmol/g pyruvate or NAC, equal to the molar amount of pyruvate in pyruvate-pretreated myocardium, was added to homogenates of untreated prearrest myocardium before extracting the enzymes. Excess pyruvate or NAC did not alter activities of any of the antioxidant-responsive enzymes (Fig. 10) or that of glucose-6-phosphate dehydrogenase, which was not affected by antioxidants before arrest. Thus residual antioxidants did not produce spurious enhancements of myocardial enzymes.

Phosphocreatine phosphorylation potential. Both antioxidant pretreatments enhanced phosphocreatine phosphorylation potential, a measure of myocardial energy state (Fig. 11). Phosphorylation potential collapsed during cardiac arrest, despite prearrest augmentation by antioxidants.

DISCUSSION

This study examined the impact of cardiac arrest and resuscitation on key enzymes of intermediary, energy, and antioxidant metabolism in left ventricular myocardium. Several enzymes were inactivated during cardiac arrest, concomitant with depletion of antioxidant reducing power. The enzyme impairments were reversible; all of the enzymes recovered during OCCC; phosphofructokinase, aconitase, malate dehydrogenase, creatine kinase, and glucose-6-phosphate dehydrogenase only recovered after ROSC. The spontaneous recovery of these enzymes indicates that neither protein degradation nor irreversible oxidative damage contributed to their inactivation during arrest. Instead, reversible modifications, e.g., sulfhydryl oxidation or thiolation, may have inactivated the enzymes.
During ischemia, phosphofructokinase is reversibly inactivated by dimerization (13) or by translocation from cytosol to membranes (21). Glyceraldehyde-3-phosphate dehydrogenase is also susceptible to oxidative modification and direct oxidant injury (26, 30). Surprisingly, this enzyme was not inactivated in this canine model of cardiac arrest and resuscitation, nor was it affected by antioxidants. Lactate dehydrogenase activity fell only marginally during arrest and was not appreciably affected by OCCC and ROSC. Similarly, Bogaert et al. (8) reported that canine brain lactate dehydrogenase is resistant to inactivation following 10 min of cardiac arrest and ROSC. The mechanism of the modest activation of lactate dehydrogenase by antioxidants is unclear.

Aconitase activity fell by 55% during cardiac arrest but recovered following OCCC and ROSC. Oxidative disassembly of its catalytic [4Fe-4S]2+ cluster inactivates aconitase, but reductants such as GSH can restore the enzyme (38). Aconitase also can be reversibly inactivated by oxidation of a sulfhydryl moiety near its active site (10). Both of these mechanisms may respond to treatment with sulfhydryl reagents such as NAC.

Citrate synthase, malate dehydrogenase, and creatine kinase are inactivated by oxidative modification and thiolation of critical cysteine residues (17, 28, 32). These enzymes are particularly susceptible to S-glutathiolation, in which glutathione disulfide, generated by oxidation of glutathione, transfers glutathionyl moieties to cysteine sulfhydryls. This modification can be reversed and the enzyme reactivated with recovery of GSH redox state. By partially preserving GSH/GSSG, pyruvate and NAC may have stabilized citrate synthase, malate dehydrogenase, and creatine kinase during arrest.

Glucose-6-phosphate dehydrogenase, the initial and rate-controlling enzyme of the hexose monophosphate shunt, is also sensitive to oxidative stress (24, 26) and is inactivated by thiol/disulfide exchange (42). After GSH is consumed to detoxify ROS, glutathione reductase regenerates the antioxidant. The sulfhydryl antioxidant NAC protected glutathione reductase during arrest without increasing its prearrest activity. On the other hand, pyruvate pretreatment, which was as effective as NAC at preserving GSH/GSSG during arrest, did not protect glutathione reductase. Arguably, inactivation of glutathione reductase may have caused the decline in GSH/GSSG during arrest, not vice versa. Glutathione reductase is inactivated by S-nitroso glutathione (3), an endogenous nitric oxide donor, and is also susceptible to catecholamine-induced oxidative stress and its inactivation is reversed by reductants (33). It therefore seems likely that oxidative stress during arrest resulted in glutathione reductase inactivation.

All of the enzymes that were inactivated during cardiac arrest spontaneously recovered by 3 h of ROSC. The reversibility of enzyme inactivation indicates that the enzymes were neither degraded nor released from injured cardiocytes; rather, the enzymes were covalently modified, and reversal of these modifications restored catalytic activity. Our recent study (35) in this model of cardiac arrest and resuscitation demonstrated that left ventricular contractile function temporarily recovered following cardioversion but then fell markedly by 2 to 3 h of ROSC. The current findings suggest that this cardiodepression was not caused by metabolic impairment and that damage to other cellular components may have contributed to the contractile dysfunction.

Unlike the other enzymes, glutathione peroxidase activity increased to well above baseline during OCCC and early ROSC, in accordance with reports of glutathione peroxidase activation in response to oxidant stress and ischemia-reperfusion (1, 12, 23). In murine fibroblasts, oxidative stress activated the tyrosine kinases c-Alb and Arg, which in turn phosphorylated and activated glutathione peroxidase (12). Prearrest activation of the enzyme by antioxidants may have resulted from increased phosphorylation potential, the major determinant of free energy of ATP hydrolysis (39), the thermodynamic driving force for protein phosphorylation. Pyruvate, an energy-generating metabolite, also increased glutathione peroxidase activity during ischemia in isolated rat hearts (15).
Glutathione redox state during cardiac arrest and recovery. Myocardial glutathione redox state (GSH/GSSG) was severely depleted at 5 min of arrest but progressively recovered during OCCC and ROSC, indicating that the major oxidative stress occurred during arrest, not reperfusion. Although oxyradical generation during ischemia is not as well characterized as the reperfusion oxidative burst (9), even during ischemia, sufficient \( \text{O}_2 \) is available in cardiomyocytes to generate ROS (5). OCCC effected only partial reperfusion of myocardium. Sequential application of OCCC, followed by cardioversion, and ROSC may have effected incremental reperfusion that dampened the oxyradical burst. Also, activation of glutathione peroxidase during OCCC and ROSC, and concomitant recovery of GSH-reducing power, may have enhanced detoxification of reactive oxygen metabolites.

Glutathione redox state recovery partially during OCCC, despite persistent inactivation of glucose-6-phosphate dehydrogenase, a major source of NADPH-reducing equivalents to regenerate glutathione from glutathione disulfide. On the other hand, full recovery of glutathione reductase during OCCC may have facilitated partial recovery of glutathione redox state, provided that sufficient NADPH was available from residual glucose-6-phosphate dehydrogenase or other sources.

NAC pretreatment augmented prearrest myocardial GSH/GSSG and partially maintained GSH/GSSG during cardiac arrest. Pyruvate did not increase prearrest myocardial GSH/GSSG, but pyruvate, like NAC, attenuated depletion of myocardial GSH/GSSG during arrest. By partially preserving GSH/GSSG, antioxidants may have protected oxidant-sensitive enzymes during cardiac arrest.

Both pyruvate and NAC markedly increased phosphocreatine-phosphorylation potential, a measure of myocardial energy state (39), before arrest. As an energy-yielding metabolic substrate, pyruvate enhances myocardial phosphorylation potential following reperfusion (11, 36) but not during ischemia (11, 24). Although NAC is not an energy-yielding fuel, antioxidants (18, 37), including NAC (25), have been found to enhance myocardial energy state. The mechanisms of antioxidant-enhancement of myocardial energetics are unclear.

Limitations. This study was performed in open-chest anesthetized dogs and therefore does not precisely mimic the conditions of closed-chest cardiac arrest and cardiopulmonary resuscitation. The open-chest preparation facilitated control of aortic pressure during OCCC and allowed heart tissue to be sampled at specific time points. This study did not identify the reactive oxygen metabolites generated during cardiac arrest or the specific protein modifications that altered enzyme activities. The possibility that antioxidants may hasten postarrest recovery of myocardial enzymes and thereby enhance postarrest energy metabolism and contractile performance remains to be tested. The GSH/GSSG system is only one of several redox systems comprising the myocardial antioxidant defenses. However, GSH/GSSG is abundant and exists in a state of near equilibrium with these other antioxidants (20, 34), so GSH/GSSG provides an integrated index of the collective status of myocardial antioxidant defenses.

In conclusion, several important myocardial metabolic enzymes are reversibly inactivated by oxidative stress during cardiac arrest. Cardiopulmonary resuscitation effected partial recovery of some of these enzymes, and all of the enzymes eventually recovered following resumption of spontaneous circulation. Pretreatments with metabolic (pyruvate) and pharmacological (NAC) antioxidants alleviated enzyme inactivation during arrest, in part by enhancing prearrest activities. These results implicate oxidative stress as a major contributor to reversible inactivation of myocardial enzymes during cardiac arrest.
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