Reversible Blockade of Electron Transport during Ischemia Protects Mitochondria and Decreases Myocardial Injury following Reperfusion

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ABSTRACT

Cardiac mitochondria sustain damage during ischemia and reperfusion, contributing to cell death. The reversible blockade of electron transport during ischemia with amobarbital, an inhibitor at the rotenone site of complex I, protects mitochondria against ischemic damage. Amobarbital treatment immediately before ischemia was used to test the hypothesis that damage to mitochondrial respiration occurs mainly during ischemia and that protection of mitochondria during ischemia leads to decreased cardiac injury with reperfusion. Langendorff-perfused Fischer-344 rat hearts were treated with amobarbital (2.5 mM) or vehicle for 1 min immediately before 25 min of global ischemia. Both groups were reperfused for 30 min without additional treatment. Subsarcolemmal (SSM) and interfibrillar (IFM) populations of mitochondria were isolated after reperfusion. Ischemia and reperfusion decreased state 3 and increased state 4 respiration rate in both SSM and IFM. Amobarbital treatment protected oxidative phosphorylation measured following reperfusion and improved the coupling of respiration. Cytochrome c content measured in SSM and IFM following reperfusion decreased in untreated, but not in amobarbital-treated, hearts. H2O2 release from SSM and IFM isolated from amobarbital-treated hearts during reperfusion was markedly decreased. Amobarbital treatment against ischemia improved recovery of contractile function (percentage of preischemic developed pressure: untreated 51 ± 4%, n = 12; amobarbital 70 ± 4%, n = 11, p < 0.01) and substantially reduced infarct size (untreated 32 ± 2%, n = 7; amobarbital 13 ± 2%, n = 7, p < 0.01). Thus, mitochondrial damage occurs mainly during ischemia rather than during reperfusion. Reperfusion in the setting of preserved mitochondrial respiratory function attenuates the mitochondrial release of reactive oxygen species, enhances contractile recovery, and decreases myocardial infarct size.

Mitochondria are both targets and sources of damage during ischemia and reperfusion (Borutaite et al., 2001; Lesnefsky et al., 2001b). Cardiac ischemia damages the mitochondrial electron transport chain (Rouslin, 1983) and decreases the rate of oxidative phosphorylation (Lesnefsky et al., 1997, 2001a). In the isolated, buffer-perfused rat heart, 25 min of global ischemia decreases the activity of complex III, the content of cytochrome c, and respiration through cytochrome oxidase (Lesnefsky et al., 2001a). Ischemic damage to mitochondria persists during reperfusion (Lesnefsky et al., 2004b), and mitochondria may sustain additional damage during the reperfusion period as well (Veitch et al., 1992; Chen et al., 2001a,b). The presence of damaged mitochondria during reperfusion decreases energy production, increases the generation of reactive oxygen species (ROS) (Ambrosio et al., 1993; Becker et al., 1999; Turrens, 2003; Becker, 2004), and augments the release of cytochrome c (Borutaite et al., 2001, 2003; Chen et al., 2001a; Borutaite and Brown, 2003; Weiss et al., 2003). Preservation of mitochondrial function is critical in diminishing myocardial injury during ischemia and reperfusion.

The production of ROS is increased in myocardium during ischemia (Becker et al., 1999; Kevin et al., 2003) as the myocardial oxygen content present during ischemia...
Phosphorylation and the content of cytochrome in a manner similar to rotenone preserves oxidative phosphorylation. Barbiturate that inhibits at the rotenone site of complex I by amobarbital treatment provides an experimental approach to separate the contribution of ischemia from that of reperfusion. Amobarbital is a short-acting barbiturate that inhibits at the rotenone site of complex I (Chance et al., 1963; Horgan et al., 1968; Degli Esposti, 1998). Inhibition of respiration through complex I by amobarbital is rapidly reversible (Spiegel and Wainio, 1969).

Amobarbital (2.5 mM) given immediately before ischemia in a manner similar to rotenone preserves oxidative phosphorylation and the content of cytochrome c in both subsarcolemmal mitochondria (SSM) and interfibrillar mitochondria (IFM) after 25 min of ischemia in the isolated rat heart (Chen et al., 2006). Thus, a reversible inhibitor of electron transport protects mitochondria, including respiration through complex I, from ischemic damage (Chen et al., 2006).

The protection of mitochondrial respiration during ischemia by amobarbital treatment provides an experimental approach to separate the contribution of ischemia from that of reperfusion in the genesis of mitochondrial damage. The finding of preserved oxidative phosphorylation after reperfusion when amobarbital reversibly inhibits electron transport during ischemia would support the belief that the bulk of mitochondrial damage occurs during ischemia, rather than during reperfusion. Preservation of mitochondrial function during reperfusion, in turn, would allow reperfusion of myocardium in the setting of preserved mitochondrial function. The finding of decreased cardiac injury in the setting of preserved mitochondrial function during reperfusion would strongly support the notion that mitochondrial damage during ischemia leads to additional myocardial damage during the early reperfusion period. This link between ischemic mitochondrial damage and myocardial injury during reperfusion provides a consistent mechanism for the myocardial protection observed with transient inhibition of mitochondrial function during reperfusion by hypoxic reperfusion (Petrosillo et al., 2005), pharmacologic inhibition (Ambrosio et al., 1993; Park et al., 1997), or brief, repetitive episodes of recurrent ischemia (Yellon and Hausenloy, 2005).

Materials and Methods
Preparation of Rat Hearts for Perfusion. The Animal Care and Use Committees of the Louis Stokes Cleveland Veterans Affairs Medical Center and Case Western Reserve University approved the protocol. Male Fisher rats [6–8 months of age (350–420 g)] were anesthetized with pentobarbital sodium (100 mg/kg i.p.) and anticoagulated with heparin (1000 IU/kg i.p.). Hearts were excised and perfused retrograde via the aorta with modified Krebs-Henseleit buffer oxygenated with 95% O2/5% CO2 at pH 7.4 at 37°C. Cardiac tissue was finely minced and placed in buffer A containing 10 mM sodium phosphate buffer using the difference of sodium concentration to separate the contribution of ischemia from that of reperfusion. Endogenous substrates were depleted by the addition of 0.1 mM ADP when glutamate was the substrate.

Oxygen consumption by mitochondria was measured using a Clark-type oxygen electrode at 30°C (Lesnfsky et al., 1997; Chen et al., 2006). Mitochondria were incubated in 80 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM MgSO4, 7 mM H2O2, and 1 mM ATP, pH 7.4 at 4°C. Cardiac mitochondria were isolated using the procedure of Palmer (1977) except that trypsin was used as the protease (Chen et al., 2006). Cardiac tissue was finely minced and placed in buffer A containing 0.2% bovine serum album and homogenized with a polytron tissue processor (Brinkman Instruments, Westbury, NY) for 2.5 s at a rheostat setting of 6.0. The polytron homogenate was centrifuged at 500g, the supernatant saved for isolation of SSM, and the pellet washed. The combined supernatants were centrifuged at 3000g to sediment SSM. IFM were isolated by incubation of skinned myofibers, obtained after polytron treatment, with 5 mg/g (wet weight) trypsin for 10 min at 4°C. SSM and IFM were washed twice and then suspended in 100 mM KCl, 50 mM MOPS, and 0.5 mM EGTA. Mitochondrial protein concentration was measured by the Lowry method, using bovine serum album as a standard.

Mitochondrial Oxidative Phosphorylation. Oxygen consumption by mitochondria was measured using a Clark-type oxygen electrode by Chen et al., 2006. Mitochondria were incubated in 80 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM MgSO4, and 1 mg of defatted, dialyzed bovine serum albumin/ml at pH 7.4. Glutamate as complex I substrate, 20 mM), succinate (complex II substrate, 20 mM), duroquinol (complex III substrate, 5 mM), and N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD)-ascorbate (complex IV substrate, 10 mM) were used, and state 4 (ADP-limited) respiration, respiratory control ratio, maximal ADP-stimulated respiration (2 mM ADP), and the ADP/O ratio were determined. Mitochondria always were used within 6 h after isolation from tissue. Endogenous substrates were depleted by the addition of 0.1 mM ADP when glutamate was the substrate.

Electron Transport Chain and Citrate Synthase Enzyme Activities. The following enzymes’ activities were measured in detergent-solubilized, freshly isolated SSM and IFM using previously described methods (Hoppel et al., 1987; Lesnfsky et al., 1997): NADH-cytochrome c reductase, rotenone-sensitive; NADH-decyldiquinone reductase, rotenone sensitive (complex I); cytochrome oxidase; and citrate synthase were measured in SSM and IFM at 37°C. Outer mitochondrial membrane integrity was assessed by measuring the rate of oxidation of exogenous reduced cytochrome c in the presence and absence of detergent. The percentage change of cytochrome oxidase activity with and without detergent reflects the integrity of the outer membrane.

Detection of H2O2 Production. H2O2 production from intact mitochondria was measured using the oxidation of the fluorogenic indicator Amplex red in the presence of horseradish peroxidase (Chen et al., 2003).

Measurement of Cytochrome Content. Cytochrome contents were determined in mitochondria solubilized in 2% deoxycholate in 10 mM sodium phosphate buffer using the difference of sodium dithionite-reduced and air-oxidized spectra (Lesnfsky et al., 2001a).

Determination of Infarct Size. Infarct size was measured in two additional groups of UTR and 2.5 mM AMO (Chen et al., 2006) hearts except that reperfusion was extended to 120 min for the measurement of infarct size as described previously (Hedayati et al., 2003); freezing at −20°C for 20 min, sectioning into 2-mm-thick
slices, incubation in 1% 2,3,5-triphenyltetrazolium chloride for 20 min at 37°C, and storage in 10% formalin followed by planimetry. Infarct size was expressed as percentage of the entire myocardium.

**Statistical Analysis.** Data are expressed as the mean ± S.E.M. Differences among groups were compared by one-way analysis of variance with post hoc comparisons performed using the Student-Newman-Keuls test of multiple comparisons. A difference of $p < 0.05$ was considered significant.

**Results**

**Reversible Blockade of Electron Transport during Ischemia Decreases Myocardial Injury Measured following Reperfusion.** In all three groups (TC, UTR, and AMO), LVDP is similar in each group at the end of equilibration perfusion (15 min) immediately before the administration of amobarbital or vehicle (Fig. 1A). Amobarbital infusion resulted in a rapid decrease in systolic and developed pressures without an increase in diastolic pressure within 1 min (data not shown). Blockade of electron transport with amobarbital during ischemia markedly attenuated the increase in diastolic pressure during ischemia (Fig. 1B). The recovery of left ventricular systolic function (Fig. 1A, LVDP; Fig. 1C, $dP/dt_{max}$) and diastolic function (Fig. 1B, diastolic pressure; Fig. 1D, $dP/dt_{min}$) during reperfusion were substantially improved by reversible blockade of electron transport during ischemia. Compared with untreated hearts, amobarbital before ischemia improved coronary flow during reperfusion (UTR: 4.5 ± 0.4 ml/min, $n = 12$; AMO: 7.9 ± 0.7, $n = 11$; TC: 9.9 ± 0.5, $n = 5$, $p < 0.05$ UTR versus others).

Reversible inhibition of respiration during ischemia significantly attenuated myocardial injury evident following ischemia and reperfusion [lactate dehydrogenase release during the initial 30 min of reperfusion; UTR: 427 ± 60 (mU/min/g), $n = 11$ versus AMO: 261 ± 22, $n = 9$, $p < 0.05$]. Amobarbital treatment to block respiration during ischemia substantially reduced myocardial infarct size measured after 120 min of reperfusion (Fig. 2).

**Reversible Blockade of Electron Transport during Ischemia Preserves Mitochondrial Function following Reperfusion.** The protein yield of SSM and IFM was not altered by amobarbital treatment (SSM: UTR 10.0 ± 0.5 mg/g, $n = 12$ versus AMO 9.8 ± 0.6, $n = 11$, $p = N.S.$; IFM: UTR 8.9 ± 0.3 versus AMO 9.3 ± 0.4, $p = N.S.$). The protein yield of each population was slightly higher in TC hearts (SSM: 13.3 ± 0.3 mg/g, $n = 5$; IFM: 11.7 ± 0.4 versus AMO 9.3 ± 0.4, $p < 0.05$). The specific activity of citrate synthase, a mitochondrial marker enzyme, was also not altered by amobarbital treatment (SSM: UTR 2260 ± 90 mU/mg protein, $n = 7$ versus AMO 2400 ± 190, $n = 7$, $p = N.S.$; IFM: UTR 3190 ± 160 versus AMO 3350 ± 200, $p = N.S.$.). The specific activity of citrate synthase was slightly decreased in SSM (1650 ± 70 mg/g, $n = 5$, $p < 0.05$) and similar in IFM from TC hearts (2600 ± 300, $n = 5$, $p = N.S.$) compared with the UTR and AMO groups.

With glutamate as a substrate to donate reducing equivalents to complex I, ischemia and reperfusion decreases the rate of state 3 and increases the rate of state 4 respiration compared with TC in both SSM and IFM (Fig. 3, A and B).

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**Fig. 1.** Left ventricular contractile function during ischemia and reperfusion. Amobarbital treatment immediately before ischemia improved the recovery of systolic function observed during reperfusion as shown by greater LVDP in A and $dP/dt_{max}$ in C (white bars, UTR; hatched bars, AMO). Amobarbital prevented ischemic contracture at 25 min of ischemia (B) and improved diastolic function during reperfusion as shown by the lower diastolic pressure in B and the increased $dP/dt_{min}$ in D. Data are expressed as mean ± S.E.M.; *, $p < 0.05$ versus nonischemic time control; †, $p < 0.05$ versus untreated ischemia and reperfusion; nonischemic time control, $n = 5$; untreated ischemia and reperfusion, $n = 12$; amobarbital treatment, $n = 11$; ISC, ischemia; REP, reperfusion.
Amobarbital treatment before ischemia markedly improved state 3 respiration in both populations of mitochondria isolated after 30 min of reperfusion compared with the UTR group (Fig. 3A). Amobarbital prevented the increase in state 4 respiration observed in untreated hearts (Fig. 3B). The preservation of state 3 and state 4 respiratory rates improved the coupling of respiration, reflected in the respiratory control ratio (Fig. 3C). Amobarbital treatment did not alter the ADP/O ratio in SSM nor IFM.

Ischemia and reperfusion decrease the rate of oxidative phosphorylation using substrates that donate electrons to complex II (succinate), complex III (duroquinol), and complex IV via cytochrome c (TMPD-ascorbate) in both SSM and IFM (Table 1), whereas amobarbital treatment preserved oxidative phosphorylation in the two populations of mitochondria with all the substrates (Table 1). Ischemia and reperfusion decreased the rate of dinitrophenol-uncoupled respiration, whereas amobarbital preserved the rate. Oxidative phosphorylation in both SSM and IFM was similar in the nonischemic TC and the ischemic and reperfused AMO group.
brane permeability is required for the loss of cytochrome c (data not shown). An increase in mitochondrial outer-membrane integrity following reperfusion (Fig. 4A). The contents of cytochromes in both SSM and IFM measured after 25 min of ischemia and from both SSM and IFM (Fig. 4A). Amobarbital treatment before ischemia main-

Thus, the reversible blockade of electron transport only during ischemia preserves mitochondrial function during the subsequent reperfusion period.

**Reversible Blockade of Electron Transport during Ischemia Preserves Complex I Activity following Reperfusion.** Complex I sustains damage during ischemia (Rouslin, 1983), as well as during reperfusion (Veitch et al., 1992). To further confirm the reversibility during reperfusion of amobarbital blockade at complex I, activity was measured in mitochondria isolated after reperfusion. NADH/cytochrome c reductase (NCR) (rotenone-sensitive) measures complex I-III activity with complex I considered to be the rate-controlling step (Hoppel et al., 1987). Ischemia and reperfusion decreased NCR activity in both SSM and IFM (Table 2). Amobarbital treatment before ischemia maintained the NCR activity in both populations of mitochondria following reperfusion, with specific activity similar to the nonischemic TC group (Table 2). Complex I activity (NADH/duroquinone reductase) also was decreased by ischemia and reperfusion and also tended to improve with amobarbital treatment and was again similar to perfusion controls (Table 2). Thus, blockade of electron transport during ischemia by amobarbital is not only fully reversible during reperfusion but also blockade during ischemia protects complex I activity measured following reperfusion.

**Reversible Blockade of Electron Transport during Ischemia Preserves Cytochrome c Content and Mitochondrial Outer-Membrane Integrity following Reperfusion.** Ischemia and reperfusion caused cytochrome c loss from both SSM and IFM (Fig. 4A). Amobarbital treatment immediately before ischemia preserved cytochrome c content in both SSM and IFM measured after 25 min of ischemia and 30 min of reperfusion (Fig. 4A). The contents of cytochromes c1, b, and aa3 were similar in TC, UTR, and AMO groups (data not shown). An increase in mitochondrial outer-membrane permeability is required for the loss of cytochrome c (Ott et al., 2002). Outer-membrane integrity was assessed as the oxidation rate of reduced cytochrome c added to mitochondria in the absence or presence of detergent solubilization. An intact outer membrane prevents the oxidation of exogenous cytochrome c by restricting access to cytochrome oxidase located in the inner membrane. Maximal cytochrome oxidase activity, measured in the presence of detergent solubilization, was similar in SSM and IFM from UTR and AMO hearts (data not shown). The oxidation of exogenous cytochrome c by intact mitochondria in the absence of detergent was increased in UTR hearts following ischemia and reperfusion compared with TC, whereas in the AMO group, the rate of oxidation was lower in both SSM and IFM (Fig. 4B).

**Reversible Blockade of Electron Transport during Ischemia Decreases Net H2O2 Production from SSM and IFM following Reperfusion.** Because horseradish peroxidase and Amplex red do not enter intact mitochondria, only H2O2 that is released from mitochondria is detected by this assay (Chen et al., 2003). Ischemia and reperfusion increased the net release of H2O2 from isolated, intact mitochondria compared with nonischemic perfusion controls with succinate as the substrate (in the presence of rotenone to prevent reverse electron flow) (Fig. 5). With glutamate as substrate, an increase in the net H2O2 release resulting from ischemia and reperfusion was not observed (data not shown). With succinate as the substrate, net H2O2 release was substantially decreased in mitochondria isolated from AMO hearts (Fig. 5). The preservation of integrated respiration in mitochondria from AMO hearts led to a decrease in the release of ROS from mitochondria obtained from reperfused myocardium.

**Discussion**

The blockade of electron transport during ischemia protects mitochondria against ischemic damage (Lesnfsky et
The ability to protect mitochondrial respiration during the ischemic period allows a critical test of the contribution of ischemic damage to mitochondria to the myocardial injury observed following ischemia and reperfusion. In the present study, reversible blockade of electron transport during ischemia with amobarbital preserved the rate and coupling of oxidative phosphorylation in both SSM and IFM following reperfusion. Furthermore, amobarbital-mediated blockade of respiration during ischemia preserved the integrity of the inner and outer mitochondrial membranes assessed after reperfusion, reflected in the retention of cytochrome c by mitochondria. When mitochondrial integrity and function were protected during ischemia (Lesnefsky et al., 2004a; Chen et al., 2006), additional mitochondrial damage during reperfusion was not observed in the current study. Mitochondrial function remained similar to the function in mitochondria isolated from nonischemic perfusion control hearts. These findings strongly support the hypothesis that mitochondrial damage occurs mainly during ischemia.

The role of ischemic mitochondrial damage in myocardial injury was then addressed. When the electron transport chain was protected against ischemic damage, the release of ROS from mitochondria during reperfusion decreased (Fig. 5). Reperfusion of the heart in the setting of preserved mitochondrial function improved contractile recovery and decreased infarct size. Thus, ischemic damage to the electron transport chain contributes to additional myocardial injury during reperfusion.
Cardiac ischemia damages the electron transport chain (Lesnefsky et al., 1997, 2001b,c). In isolated rat hearts, 25 min of ischemia decreased oxidative phosphorylation in both SSM and IFM (Lesnefsky et al., 2001a; Chen et al., 2006). In the present study, ADP-stimulated respiration decreased in SSM and IFM after 30 min of reperfusion. A corresponding decrease in uncoupled respiration indicated that damage occurred in the electron transport chain rather than in the phosphorylation apparatus. Decreased ADP-stimulated and -uncoupled respiration with duroquinol as substrate following ischemia supports the presence of damage to the distal electron transport chain (Lesnefsky et al., 1997, 2001a,c). Ischemic damage to the distal electron transport chain involves complex III (Lesnefsky et al., 2001a), cytochrome oxidase (Lesnefsky et al., 1997, 2001c), and a decrease in cytochrome c content (Lesnefsky et al., 1997, 2001a,c). Amobarbital is a short-acting barbiturate anesthetic agent that inhibits complex I (Degli Esposti, 1998) by binding at the rotenone site (Horgan et al., 1968) that at low millimolar concentrations selectively inhibits respiration at complex I (Hatefi, 1968) and is rapidly reversible (Spiegel and Wainio, 1969). Amobarbital blockade attenuates electron flow into complex III and protects against damage to the distal electron transport chain assessed at the end of ischemia (Chen et al., 2006). In the current study, protection of the distal electron transport chain by reversible blockade of electron transport during ischemia was carried forward into the reperfusion period, with preserved respiration through cytochrome c by mitochondria (Fig. 4), and enhanced respiration with duroquinol as an electron donor to complex III (Table 1). Reversible blockade of electron transport with amobarbital at the rotenone site of complex I also protected complex I itself, shown by preserved rates of glutamate respiration (Fig. 3; Table 1) and complex I enzyme activity (Table 2). Thus, blockade of electron transport protects against ischemic damage to multiple sites within the electron transport chain, reflected in the preservation of oxidative phosphorylation in intact mitochondria (Fig. 3; Table 1).

State 4 respiration, an index of mitochondrial inner membrane permeability, is increased in both SSM and IFM following ischemia (Lesnefsky et al., 2001a; Chen et al., 2006). State 4 rates remained elevated following reperfusion (Fig. 3), consistent with a persistent increase in inner membrane permeability. Enhanced inner membrane permeability favors proton leak back to the mitochondrial matrix without passing through complex V, compromising ATP production (Lesnefsky et al., 2001b). Blockade of electron transport during ischemia with amobarbital attenuated the increase in state 4 respiration observed following reperfusion, indicative of a relative preservation of inner membrane integrity. Taken together, amobarbital-mediated increases in state 3 respiration, decreases in state 4 respiration, and improved respiratory control ratio increase the efficiency of oxidative phosphorylation and favor high energy phosphate production during reperfusion.

Cytochrome c is localized at the inner membrane by non-ionic and electrostatic interactions (Ott et al., 2002; Iverson and Orrenius, 2004). Ischemic damage to the inner membrane favors the delocalization of cytochrome c from the inner membrane, the first step leading to cytochrome c release from mitochondria (Lesnefsky et al., 2001c; Ott et al., 2002). The outer membrane is normally impermeable to cytochrome c (Borutaite and Brown, 2003; Weiss et al., 2003). Delocalized cytochrome c can be released from mitochondria when outer-membrane permeability increases (Ott et al., 2002; Weiss et al., 2003). In the present study, outer-membrane permeability increased following ischemia and reperfusion. Blockade of electron transport during ischemia preserved outer-membrane integrity measured after reperfusion. Preserved outer-membrane integrity is consistent with the improved retention of cytochrome c by mitochondria isolated from AMO hearts.

Myocardial ischemia initiates the onset of apoptosis (Borutaite and Brown, 2003; Borutaite et al., 2003) that accelerates during reperfusion (Borutaite et al., 2001, 2003; Chen et al., 2001b; Borutaite and Brown, 2003). Cytochrome c loss from mitochondria is a key amplifying step of the apoptotic cascade during ischemia and reperfusion (Borutaite et al., 2001; Chen et al., 2001b). In addition, cytochrome c loss inhibits respiration (Lesnefsky et al., 1997, 2001b,c) and increases the generation of ROS (Kushnareva et al., 2002; Ricci et al., 2003; Chen and Lesnefsky, 2006). Thus, preservation of cytochrome c content is a key mechanism in protecting myocardium during ischemia and reperfusion (Lesnefsky et al., 2001c, 2004a; Borutaite et al., 2003).

Amobarbital treatment immediately before ischemia prevented the increase in diastolic pressure during ischemia (Fig. 1B). In our previous study, administration of the complex I inhibitor rotenone immediately before ischemia protected mitochondria against ischemic damage without affecting the increase of diastolic pressure during ischemia (Lesnefsky et al., 2004a). This finding does not support a link between ischemic contracture and mitochondrial protection during ischemia.

Reperfusion of myocardium in the absence of ischemic damage to the electron transport chain led to improved contractile recovery (Fig. 1) and decreased infarct size (Fig. 2). The observations of the current study that damage to the electron transport chain occurs mainly during ischemia, coupled with the observed cardiac protection when the heart is reperfused in the setting of preserved mitochondrial function, provide strong support for the hypothesis that ischemic damage to mitochondria is a key mechanism of myocardial injury during reperfusion. Previous approaches have attempted to protect reperfused myocardium from the ischemic mitochondrial damage that had already occurred by transiently inhibiting mitochondrial function during reperfusion by pharmacologic (Ambrosio et al., 1993; Park et al., 1997), hypoxic (Petrosillo et al., 2005), or even additional ischemic (Yellon and Hausenloy, 2005) interventions. Blockade of electron transport during ischemia provides a new mechanistic concept concerning the prevention of mitochondrial damage during ischemia to decrease subsequent mitochondrial-driven cardiac injury during reperfusion.

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References


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