Blockade of Electron Transport before Cardiac Ischemia with the Reversible Inhibitor Amobarbital Protects Rat Heart Mitochondria

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ABSTRACT

Cardiac ischemia damages the mitochondrial electron transport chain. Irreversible blockade of electron transport at complex I by rotenone decreases ischemic damage to cardiac mitochondria by decreasing the loss of cytochrome c and preserving respiration through cytochrome oxidase. Therapeutic intervention to protect myocardium during ischemia and reperfusion requires the use of a reversible inhibitor that allows resumption of oxidative metabolism during reperfusion. Amobarbital is a reversible inhibitor at the rotenone site of complex I. We asked whether amobarbital administered immediately before ischemia protected respiratory function. Isolated rat hearts were perfused for 15 min followed by 25-min global ischemia at 37°C. Amobarbital-treated hearts received drug for 1 min before ischemia. Subsarcolemmal (SSM) and interfibrillar (IFM) populations of mitochondria were isolated after ischemia, and oxidative phosphorylation was measured. Amobarbital protected oxidative phosphorylation, including through cytochrome oxidase, in both SSM and IFM in a dose-dependent manner, with an optimal dose of 2 to 2.5 mM. Amobarbital also preserved cytochrome c content in both SSM and IFM. Thus, reversible blockade of the electron transport chain during ischemia protects mitochondrial respiration.

Mitochondrial dysfunction contributes to myocardial injury during ischemia and reperfusion (Borutaite et al., 2001; Lesnefsky et al., 2001b; Lesnefsky and Hoppel, 2003). Ischemia damages the electron transport chain leading to decreased rates 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, content of cytochrome c, and oxidation through cytochrome oxidase in both subsarcolemmal mitochondria (SSM), located beneath the plasma membrane, and interfibrillar mitochondria (IFM) present between the myofibrils (Lesnefsky et al., 2001a). Damage to the electron transport chain occurs mainly during ischemia rather than reperfusion (Lesnefsky et al., 2004b).

Mitochondria are a source of reactive oxygen species during ischemia that contribute to myocardial injury (Becker et al., 1999; Kevin et al., 2003). Blockade of electron transport by rotenone, an irreversible inhibitor of complex I, decreased the production of reactive oxygen species from isolated mitochondria (Chen et al., 2003) and protected cardiomyocytes during simulated ischemia (Becker et al., 1999). In isolated rabbit hearts, treatment with the irreversible inhibitor of respiration rotenone immediately before in situ ischemia markedly attenuated damage to the distal electron transport chain (Lesnefsky et al., 2004a). Taken together, these findings support that electron transport is a key source of mitochondrial damage during ischemia. Since most mitochondrial damage appears to occur during ischemia (Lesnefsky et al., 2004b), we propose that if protection of oxidative function during ischemia can be achieved with reversible inhibition of electron transport, then reperfusion in the setting of preserved mitochondrial function is a feasible goal. An inhibitor of electron transport similar to rotenone but reversible that protects mitochondrial function during ischemia is required.

Amobarbital (Amytal) is a short-acting barbiturate anesthetic agent that inhibits complex I between flavoprotein and ubiquinone (Degli Esposti, 1998). Inhibition of respiration with amobarbital induces a “crossover point” leading to re-

ABBREVIATIONS: SSM, subsarcolemmal mitochondria; IFM, inter fibrillar mitochondria; MOPS, 4-morpholinepropanesulfonic acid; TMPD, N,N,N′,N′-tetramethyl p-phenylenediamine.
duction of NADH and oxidation of cytochrome b, consistent with a site of inhibition distal in the path of electron flow through complex I (Chance et al., 1963). Amobarbital competes for binding to complex I with rotenone, indicating binding at the “rotenone-site” of complex I (Horgan et al., 1968). Consistent with inhibition of complex I, amobarbital at low millimolar concentrations inhibited respiration with glutamate, whereas succinate respiration was unaffected (Ernster et al., 1955; Hatefi, 1968). The inhibition of respiration through complex I by amobarbital is rapidly reversible (Spiegel and Wainio, 1969). Consistent with reversible inhibition of mitochondrial respiration, administration during early reperfusion improved contractile recovery measured later in reperfusion in isolated rabbit (Ambrosio et al., 1993) and rat (Park et al., 1997) hearts, indicating that amobarbital can be administered to the isolated heart and will “wash out” with resumption of aerobic metabolism (Ambrosio et al., 1993) and contractile function (Ambrosio et al., 1993; Park et al., 1997). In these studies, amobarbital was used to attenuate the mitochondrial-derived cardiac injury during reperfusion that likely resulted from pre-existing ischemic mitochondrial damage (Lesnefsky et al., 2001a,b, 2004a,b; Borutaite and Brown, 2003; Turrens, 2003). Based on previous work (Lesnefsky et al., 2004a), to minimize ischemic damage, amobarbital must be administered immediately before ischemia. In the present study, reversible inhibition of electron transport with amobarbital was used to evaluate whether reversible blockade of electron transport immediately before ischemia protects oxidative phosphorylation. Next, the optimal dose of amobarbital was established. Amobarbital protected oxidative phosphorylation in both SSM and IFM in a dose-dependent manner, with an optimal dose of 2 to 2.5 mM. Based on the findings of the current study, the hypothesis that reperfusion with preserved mitochondrial function limits myocardial injury can now be tested. Protection of mitochondrial respiration with amobarbital will allow the separation of ischemic mitochondrial damage from that occurring with reperfusion.

Materials and Methods

Isolated Rat Heart Model of Ischemia and Reperfusion (Lesnefsky et al., 2001a). The Animal Care and Use Committees of the Louis Stokes Veterans Affairs Cleveland Medical Center and Case Western Reserve University approved the protocol. Adult male Fisher 6- to 8-month old rats (350–420 g) were obtained from the National Institute of Aging colony (Harlan, Indianapolis, IN). Rats were anticoagulated with heparin (1000 IU/kg i.p.) and then anesthetized with pentobarbital sodium (100 mg/kg i.p.). Hearts were excised and perfused on Langendorff apparatus with modified Krebs-Henseleit buffer oxygenated with 95% O2/5% CO2 (pH 7.35–7.45) (115 mM NaCl, 4.0 mM KCl, 1.2 mM MgSO4, 0.9 mM KH2PO4, 22.5 mM NaHCO3, 2.5 mM CaCl2, and 5.5 mM glucose). Left ventricular pressure was measured using a balloon inserted into the left ventricle. Diastolic pressure was adjusted to 5 to 10 mm Hg during equilibration. Hearts were paced at 300 beats/min. Pacing was discontinued during amobarbital infusion and during ischemia. Ischemia was induced by stopping flow. Temperature was maintained at 37°C during ischemia by placing the heart in a water-jacketed chamber and confirmed by temperature probe within the left ventricle. During ischemia, the hearts become asystolic. Untreated ischemic hearts were subjected to 25-min global ischemia. Ischemic contracture was measured as the time to a 5 mm Hg increase of diastolic pressure during ischemia (Grover et al., 2001). If left ventricular pressure rose by less than 5 mm Hg during ischemia, 25 min was used as the time to contracture. In amobarbital-treated ischemic hearts, amobarbital (1.25, 2.5, 3.75, and 5 mM) in oxygenated Krebs-Henseleit buffer was infused for 1 min immediately before induction of ischemia. Time control hearts were perfused for 41 min in the absence of ischemia (Fig. 1).

Isolation of Two Populations of Cardiac Mitochondria. At the end of ischemia, hearts were removed from perfusion column and placed into buffer A (100 mM KCl, 50 mM MOPS, 5 mM EGTA, 5 mM MgSO4, 7 mM H2O, and 1 mM ATP; pH 7.4) at 4°C. Cardiac mitochondria were isolated according to Palmer et al., with minor modifications (Palmer et al., 1977; Moghadass et al., 2002). Cardiac tissue was finely minced and placed in buffer A containing 0.2% bovine serum albumin and homogenized with a polytorn tissue processor (Brinkman Instruments, Westbury, NY) for 2.5 s at a 250 rpm homogenization speed. The homogenate was centrifuged at 500g the supernatant was saved for isolation of SSM, and the pellet was washed. The combined supernatants were centrifuged at 3000g to sediment SSM. IFM were isolated by incubation of skinned myofibers, obtained following 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 KME (100 mM KCl, 50 mM MOPS, and 0.5 mM EGTA). Mitochondrial protein concentration was determined by the Lowry method, using bovine serum albumin as a standard (Lowry et al., 1951).

Mitochondrial Oxidative Phosphorylation. Oxygen consumption in mitochondria was measured using a Clark-type oxygen elec-
trode at 30°C. Mitochondria were incubated in a solution containing 80 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM KH₂PO₄, and 1 mg/ml bovine serum albumin, at pH 7.4. Glutamate (complex I substrate), succinate (complex II substrate), duroquinol (complex III substrate), and TMPD-ascorbate (complex IV substrate) were used as electron donors to specific sites in the electron transport chain, and state 3 (ADP-stimulated), state 4 (ADP-limited) respiration, respiratory control ratios, and the ADP/O ratio were measured.

Measurement of Citrate Synthase and Cytochrome Contents. Citrate synthase activity was quantified in cholate-solubilized mitochondria by measuring the rate of 5,5'-dithiobis(nitrobenzoic acid)-reactive reduced coenzyme A (412 nm, ε = 13,600 M/cm) at 37°C as previously described (Srere, 1969). Cytochrome contents were measured in mitochondria solubilized in 2% deoxycholate in 10 mM sodium phosphate buffer. Using the difference of sodium dithionite reduced and air-oxidized spectra (Williams, 1964; Lesnefsky et al., 2001a).

Statistical Analysis. Data are expressed as the mean ± standard error of the mean. 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 (SigmaStat for Windows Version 1.0; SPSS Inc., Chicago, IL).

Results

Amobarbital Treatment before Ischemia Prevents Ischemic Contracture. In the time control group, left ventricular developed pressure, the first derivative of the rise and fall of developed pressure (positive and negative dP/dt), and coronary flow were maintained during 41 min of continuous perfusion (left ventricular developed pressure 123 ± 4 mm Hg, 15-min equilibration versus 125 ± 4 mm Hg, 41-min perfusion, n = 6, p = N.S.; other results not shown). There were no differences in hemodynamic data between time control, untreated ischemia, and amobarbital-treated ischemia groups at the end of the 15-min equilibration period before the infusion of amobarbital (data not shown). Infusion of amobarbital (each concentration) led to cessation of cardiac contraction within 30 s of infusion. In untreated ischemia, diastolic pressure increased, whereas amobarbital treatment (2 or 2.5 mM) markedly attenuated the rise of diastolic pressure during ischemia (Fig. 2). Amobarbital treatment prolonged the time to ischemic contracture, again with optimal protection provided 2 or 2.5 mM (Fig. 2).

Mitochondrial Protein Yield and Citrate Synthase Activity. The protein yield of SSM and IFM following 25 min of untreated ischemia is similar to the protein yield of each population in the time control group (Table 1). The yields of SSM and IFM were not altered by amobarbital treatment (Table 1). Citrate synthase, a mitochondrial matrix enzyme, was used as a marker enzyme for mitochondria. The specific activity of citrate synthase was not altered by ischemia nor by amobarbital treatment in either population of mitochondria (Table 1). Thus, following ischemia, there is an unchanged protein yield of mitochondria comprised of mitochondria with similar marker enzyme activity, consistent with a similar recovery of mitochondria from ischemic and time control hearts.

Amobarbital Treatment Protects Oxidative Phosphorylation during Ischemia in SSM and IFM in a Dose-Dependent Manner. Twenty-five minutes of ischemia decreases the maximal rate of ADP-stimulated respiration in both SSM and IFM with glutamate, succinate, duroquinol, and TMPD-ascorbate as substrates in isolated rat hearts (Figs. 3 and 4) as previously described (Lesnefsky et al., 2001a). In the current study, the maximal ADP-stimulated rate of oxidative phosphorylation was used as the primary experimental endpoint to evaluate whether amobarbital treatment before ischemia can attenuate ischemic damage. Amobarbital treatment immediately before ischemia protected oxidative phosphorylation in both SSM and IFM in a dose-dependent fashion. Two and 2.5 mM amobarbital were the optimal doses for mitochondrial protection.

Twenty-five minutes of ischemia decreased respiration that was uncoupled by the addition of dinitrophenol, localizing ischemic damage to the electron transport chain rather than the phosphorylation apparatus (Table 2). Amobarbital treatment preserved the rate of uncoupled respiration in both SSM and IFM following ischemia, indicating that the respiratory chain was protected (Table 2).

Amobarbital treatment immediately before ischemia protected ADP-stimulated and uncoupled respiration with glutamate as substrate (Table 2; Figs. 3 and 4). The decrease in respiration following ischemia in untreated hearts with duroquinol as substrate localizes a component of ischemic damage distal to complex I (Figs. 3 and 4). The preservation of respiration with duroquinol and TMPD-ascorbate as substrates indicates that amobarbital treatment protects against damage to the distal electron transport chain. Amobarbital treatment markedly attenuates cytochrome c loss from SSM and IFM during ischemia (Fig. 5). The contents of the remaining cytochromes were not altered by ischemia nor by amobarbital treatment (Table 3).
Amobarbital treatment immediately before ischemia protects oxidative phosphorylation and preserves cytochrome c content in SSM and IFM during ischemia. The protection is concentration-dependent, with 2 and 2.5 mM providing optimal mitochondrial protection. Because damage to the respiratory chain occurs mostly during ischemia rather than during reperfusion (Lesnefsky et al., 2004b), attenuation of ischemic damage is likely to preserve mitochondrial function during early reperfusion as well.

Blockade of electron transport with the irreversible complex I inhibitor rotenone protects oxidation through cytochrome oxidase and preserves cytochrome c content in the isolated rabbit heart (Lesnefsky et al., 2004a). However, glutamate oxidation is markedly inhibited since rotenone is an irreversible inhibitor of complex I (Chance et al., 1963). Rotenone treatment could not be used to study whether blockade of electron transport during ischemia also preserved respiration when electron donors to complex I were substrates for respiration. Amobarbital treatment preserved both oxidation through cytochrome oxidase, as previously observed with rotenone treatment, as well as oxidative phosphorylation with glutamate as substrate. This finding indicates that amobarbital washed out of the mitochondria dur-

## Table 1

<table>
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<tr>
<th>Protein yield (mg/g wet tissue) and the activities of citrate synthase (mU/mg of protein) of SSM and IFM following ischemia with and without amobarbital treatment</th>
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<tr>
<td>Data are expressed as mean ± S.E.M. The protein yield (milligrams of mitochondrial protein per gram wet weight of heart) and citrate synthase activities (mU/milligrams of mitochondrial protein) were not altered either by ischemia alone or by amobarbital treatment in SSM and IFM (all data, p = N.S.).</td>
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<tr>
<td><strong>Protien Content</strong></td>
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<td>---------------------</td>
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<tr>
<td><strong>SSM</strong></td>
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<tr>
<td>TIME CONTROL (n = 6)</td>
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<td>ISCHEMIA (n = 5)</td>
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<tr>
<td>1.25 mM Amobarbital (n = 3)</td>
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<td>2.0 mM Amobarbital (n = 3)</td>
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<td>2.5 mM Amobarbital (n = 5)</td>
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<tr>
<td>3.75 mM Amobarbital (n = 3)</td>
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<td>5.0 mM Amobarbital (n = 3)</td>
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## Subsarcolemmal Mitochondria

![Fig. 3](image)

Amobarbital treatment immediately before ischemia protects oxidative phosphorylation and preserves cytochrome c content in SSM and IFM during ischemia. The protection is concentration-dependent, with 2 and 2.5 mM providing optimal mitochondrial protection. Because damage to the respiratory chain occurs mostly during ischemia rather than during reperfusion (Lesnefsky et al., 2004b), attenuation of ischemic damage is likely to preserve mitochondrial function during early reperfusion as well.

## Discussion

Amobarbital treatment immediately before ischemia protects oxidative phosphorylation and preserves cytochrome c content in SSM and IFM during ischemia. The protection is concentration-dependent, with 2 and 2.5 mM providing optimal mitochondrial protection. Because damage to the respiratory chain occurs mostly during ischemia rather than during reperfusion (Lesnefsky et al., 2004b), attenuation of ischemic damage is likely to preserve mitochondrial function during early reperfusion as well.

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ing the process of mitochondrial isolation, confirming the reversible nature of inhibition of electron transport by amobarbital. Amobarbital avidly binds to albumin (Chance et al., 1963), and the use of isolation buffers containing bovine serum albumin at several steps during mitochondrial isolation (Palmer et al., 1977; Fannin et al., 1999; Moghaddas et al., 2002) provides a large protein sink for the redistribution of amobarbital from mitochondria. In contrast to the observations with amobarbital, when rotenone, an irreversible inhibitor of complex I, was administered to the heart immediately before in situ ischemia, glutamate respiration was blocked (Lesnefsky et al., 2004a). Taken together, these results indicate that amobarbital treatment immediately before ischemia results in reversible inhibition of respiration. This finding is consistent with previous observations in isolated mitochondria (Spiegel and Wainio, 1969) and in the isolated heart (Ambrosio et al., 1993; Park et al., 1997), that amobarbital can rapidly wash out following cessation of administration. Thus, it is feasible to use amobarbital to address the question whether protection of the respiratory chain against ischemic damage by reversible inhibition of electron transport is carried forward into reperfusion when electron transport resumes.

Amobarbital treatment protected against ischemia-induced decreases in glutamate respiration, demonstrating that reversible blockade of respiration at complex I protects against the ischemic damage previously reported to involve complex I (Rouslin 1983, Veitch et al., 1992) Ischemia decreases complex III activity, cytochrome c content, and respiration through cytochrome oxidase in both SSM and IFM in the distal electron transport chain (Lesnefsky et al., 2001a,b). The preservation of respiration with duroquinol and TMPD-ascorbate as substrates for respiration as well as the preserved content of cytochrome c indicates that amobarbital treatment protects against damage to the distal electron transport chain as well.

Reactive oxygen species contribute to myocardial injury

Fig. 4. Maximal rates of oxidative phosphorylation in IFM with different electron donors is shown. Ischemia decreased the maximal rate of oxidative phosphorylation stimulated with 2 mM ADP for the substrates glutamate (complex I), succinate (complex II), duroquinol (DHQ) (complex III), and TMPD-ascorbate (complex IV). Two and 2.5 mM amobarbital given before ischemia provided optimal preservation of ADP-stimulated respiration with each substrate. Mean ± S.E.M.; * p < 0.05 versus time control; † p < 0.05 versus untreated ischemic group. n = 6 for time control, and n = 5 for untreated ischemic group; n = 3 for each amobarbital group except for 2.5 mM amobarbital group, n = 5; for DHQ and TMPD-ascorbate, n = 2 for the 5 mM amobarbital group.

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<th>TABLE 2</th>
<th>Uncoupled respiration with dinitrophenol in SSM and IFM following ischemia with and without amobarbital treatment</th>
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<td></td>
<td>Data are expressed as mean ± S.E.M. Ischemia decreased uncoupled respiration in both SSM and IFM, whereas 2.5 mM amobarbital treatment before ischemia preserved the rate of uncoupled respiration.</td>
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<tr>
<td></td>
<td>SSM</td>
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<tr>
<td>Glutamate</td>
<td>DHQ</td>
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<tr>
<td>Time control (n = 6)</td>
<td>200 ± 7</td>
</tr>
<tr>
<td>Ischemia (n = 5)</td>
<td>115 ± 18</td>
</tr>
<tr>
<td>2.5 mM Amytal (n = 5)</td>
<td>228 ± 14</td>
</tr>
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Amytal, amobarbital. * P < 0.05 vs. time control; † P < 0.05 vs. ischemia.
during ischemia and reperfusion (Turrens, 2003; Becker, 2004), with the mitochondrial electron transport chain a major source of reactive oxidants (Turrens, 2003). Blockade of electron transport by rotenone protects mitochondria against ischemic damage indicating that the respiratory chain is a key source of mitochondrial damage during ischemia (Lesnefsky et al., 2004a). The finding in the current study that reversible inhibition of electron transport during ischemia protects mitochondria provides further support for this concept. Ischemic damage to electron transport leads to increased oxidant generation in rabbit heart submitochondrial particles with NADH as substrate (Chen et al., 2004). Thus, ischemic damage to the electron transport chain facilitates oxyradical generation and sets the stage for myocardial injury during reperfusion. Preserved function of the electron transport chain after ischemia is likely to decrease oxidant generation and minimize myocardial injury during reperfusion.

Complexes I and III are the major sites for oxyradical generation (Gille and Nohl, 2001; Lesnefsky et al., 2001b; Chen et al., 2003; Han et al., 2003; Turrens, 2003). Blockade of electron transport at the rotenone site of complex I with amobarbital would be predicted to increase superoxide generation and worsen mitochondrial function following ischemia. However, rotenone (Lesnefsky et al., 2004a), or in the current study amobarbital, protected mitochondria. These results do not support that complex I is the major site for cytotoxic oxidant generation in intact mitochondria during ischemia. Indeed, rotenone treatment of isolated, intact mitochondria did not significantly increase net H$_2$O$_2$ generation in the presence of complex I substrates (Chen et al., 2003). In contrast, inhibition of complex III with antimycin A markedly enhanced H$_2$O$_2$ production from mitochondria with complex I substrates (Gille and Nohl, 2001; Chen et al., 2003; Han et al., 2003) that was attenuated when rotenone was also present (Chen et al., 2003). Oxyradical release from complexes I and III are oriented in different directions with respect to the inner mitochondrial membrane. Oxidants generated by complex I are directed toward the mitochondrial matrix and likely inactivated by matrix antioxidant systems, whereas oxyradicals generated by the quinol oxidation site of complex III are released into the intermembrane space (Chen et al., 2002).

![Figure 5](image-url) Ischemia decreased cytochrome c content in both SSM and IFM. Amobarbital (2.5 mM) given before ischemia preserved cytochrome c content following ischemia. Mean ± S.E.M.; *, *p < 0.05 versus time control; †, †*p < 0.05 versus untreated ischemic group, n = 6 for time control, and n = 5 for untreated ischemic group and for 2.5 mM amobarbital group.

<table>
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<tr>
<th>TABLE 3</th>
<th>Cytochrome content in SSM and IFM following ischemia with and without amobarbital treatment</th>
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<td>Data are expressed as mean ± S.E.M. The contents of c$<em>1$, b, and a$</em>{a3}$ were not altered by ischemia either in the presence or absence of 2.5 mM amobarbital (all data <em>p</em> = N.S.). Time control, <em>n</em> = 6; ischemia, <em>n</em> = 5; and 2.5 mM amobarbital + ischemia, <em>n</em> = 5.</td>
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<tr>
<td></td>
<td>SSM (nmol/mg protein)</td>
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<tr>
<td></td>
<td>c$_1$</td>
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<tr>
<td>Time control</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Ischemia</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>Amobarbital plus ischemia</td>
<td>0.15 ± 0.01</td>
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</table>
In cardiomyocytes, rotenone treatment markedly decreases oxidant formation during simulated ischemia (Becker et al., 1999). Thus, prevention of electron flow into complex III via inhibition of complex I protects mitochondrial function and decreases oxidant generation.

Myocardial ischemia increases apoptosis (Borutaite and Brown, 2003; Borutaite et al., 2003), which becomes increasingly evident during reperfusion (Borutaite et al., 2001; Chen et al., 2001). Cytochrome c loss from mitochondria facilitates apoptosis (Borutaite et al., 2001; Chen et al., 2001). Ischemic preconditioning decreases apoptosis by preventing cytochrome c release from mitochondria (Granville and Gottlieb, 2002). Cytochrome c loss not only augments apoptosis but also inhibits respiration, leading to further increases in oxyradical generation following cytochrome c loss (Kushnareva et al., 2002; Ricci et al., 2003; Chen et al., 2004). Oxidative damage to mitochondrial electron transport, in turn, leads to the cytochrome c loss from isolated mitochondria (Ott et al., 2002; Petrosillo et al., 2003), potentially creating a vicious cycle of mitochondrial-driven oxidative injury leading to activation of programmed cell death pathways. In the current study, amobarbital treatment before ischemia preserves cytochrome c content in SSM and IFM, perhaps by inhibition of oxidant production during ischemia.

Protection of state 3 respiration by amobarbital was clearly concentration-dependent (Figs. 3 and 4). Protection of the heart against ischemic “contracture”, the rise in diastolic pressure during ischemia, strongly correlated with concentrations of amobarbital that protected mitochondrial function (Fig. 2). The suboptimal protection achieved with 1.25 mM amobarbital is consistent with an inadequate dose of drug. The cause of the suboptimal protection achieved by the 3.75 and 5 mM doses of amobarbital is less clear. Activation or blockade of alternative metabolic pathways by amobarbital has not been described. Of interest, Chance et al. (1963) found that at concentrations of amobarbital approaching 5 mM, amobarbital also inhibits succinate respiration and complex V. The concentrations of amobarbital that provide optimal protection against ischemic damage to oxidative phosphorylation in the current study are the concentrations previously found to selectively inhibit complex I. Although the mechanism for suboptimal protection of the higher doses remains unclear, the dose response in the current study raises the possibility that previous work using 5 mM doses of amobarbital is consistent with an inadequate dose of drug.

Acknowledgments

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References


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