Blockade of Electron Transport during Ischemia Protects Cardiac Mitochondria*

Edward J. Lesnefsky†‡§, Qun Chen‡, Shadi Moghaddas‡, Medhat O. Hassan, Bernard Tandler**, and Charles L. Hoppel†‡§§

From the Departments of Medicine, Divisions of ‡Cardiology, ‡‡Clinical Pharmacology, and §§Pharmacology, **School of Dentistry, Case Western Reserve University, Cleveland, Ohio 44106 and §School of Pathology and Laboratory Medicine Services, Louis Stokes Veterans Affairs Medical Center, Cleveland, Ohio 44106

Subsarcolemmal mitochondria sustain progressive damage during myocardial ischemia. Ischemia decreases the content of the mitochondrial phospholipid cardiolipin accompanied by a decrease in cytochrome c content and a diminished rate of oxidation through cytochrome oxidase. We propose that during ischemia mitochondria produce reactive oxygen species at sites in the electron transport chain proximal to cytochrome oxidase that contribute to the ischemic damage. Isolated, perfused rabbit hearts were treated with rotenone, an irreversible inhibitor of complex I in the proximal electron transport chain, immediately before ischemia. Rotenone pretreatment preserved the contents of cardiolipin and cytochrome c measured after 45 min of ischemia. The rate of oxidation through cytochrome oxidase also was improved in rotenone-treated hearts. Inhibition of the electron transport chain during ischemia lessens damage to mitochondria. Rotenone treatment of isolated subsarcolemmal mitochondria decreased the production of reactive oxygen species during the oxidation of complex I substrates. Thus, the limitation of electron flow during ischemia preserves cardiolipin content, cytochrome c content, and the rate of oxidation through cytochrome oxidase. The mitochondrial electron transport chain contributes to ischemic mitochondrial damage that in turn augments myocyte injury during subsequent reperfusion.

Mitochondria play a cardinal role in myocardial injury during ischemia and reperfusion (1–4). Ischemia leads to progressive damage to the electron transport chain (5–10). Initially, brief periods of ischemia damage complex I in the proximal electron transport chain (7, 9) followed by damage to complex III (11). As ischemic periods are lengthened to 30 and 45 min, oxidative phosphorylation through cytochrome oxidase decreases, indicating damage to the distal electron transport chain (8, 12–15). The progression of ischemic damage is more rapid in subsarcolemmal mitochondria (SSM)† located beneath the plasma membrane than in interfibrillar mitochondria (IFM) present between the myofibrils (14, 16, 17). Specifically, in the isolated rabbit heart, 30 and 45 min of ischemia decreases oxidation through cytochrome oxidase in SSM, whereas IFM remain unaffected (12, 18).

Cytochrome oxidase, located in the inner mitochondrial membrane (2, 19), is composed of 13 subunits (20). This complex requires the integrity of catalytic subunits (21) and of regulatory and structural subunits (22) as well as an intact inner mitochondrial membrane environment enriched in the phospholipid cardiolipin for optimal enzyme activity (23, 24). Ischemia does not lead to functional inactivation of a subunit peptide of cytochrome oxidase (12). The decrease in oxidation through cytochrome oxidase occurs concomitant with a selective decrease in the content of cardiolipin (18). A decrease in the cytochrome c content of SSM occurs as the cardiolipin content decreases in these organelles (18).

Mitochondria generate cytotoxic reactive oxygen species during ischemia (2, 19, 25). Cardiolipin, required for cytochrome oxidase activity (23, 24), is enriched in oxidatively sensitive linoleic acyl groups (26). We propose that during ischemia, oxidant production from sites upstream of cytochrome oxidase (possibly from complex III (27)) leads to depletion of cardiolipin, in turn leading to loss of cytochrome c and decreased rates of oxidation through cytochrome oxidase. In this event, the blockade of electron flow during ischemia should interrupt this sequence. To address this proposal during in situ ischemia in the intact heart, the electron transport chain was inhibited during ischemia and the content of cardiolipin, cytochrome c, and the rate of oxidation through cytochrome oxidase were measured following 45 min of global ischemia in the isolated rabbit heart. Treatment immediately before ischemia with rotenone, which is a potent and irreversible inhibitor of complex I, preserved cardiolipin content, cytochrome c content, and oxidation through cytochrome oxidase in SSM. Blockade of electron transport during ischemia protects against damage to more distal sites in the electron transport chain. In isolated mitochondria, rotenone blockade of electron transport decreased the production of reactive oxygen species (27). Thus, the proximal electron transport chain mediates damage to the distal electron transport chain during the progression of ischemia. Ischemic damage to mitochondria in turn leads to myocyte damage during reperfusion (2).

MATERIALS AND METHODS
All chemicals were of highest commercial purity and were obtained from Sigma. HPLC grade solvents were obtained from Fischer Scientific.
Rotenone Prevents Ischemic Damage

Table I  
Protein yield and respiration rate in myocardial mitochondria isolated from rabbit hearts with and without rotenone treatment

|                          | Non-ischemia (n = 8) | 45 minutes ischemia (n = 10) | 45 minutes ischemia-rotenone treated (n = 8) |
|--------------------------|----------------------|------------------------------|---------------------------------------------|
| Mitochondrial protein yield |                      |                              |                                             |
| SSM                      | 15.4 ± 0.5 mg of protein/ mg of wet weight | 13.9 ± 0.8 mg of protein/ mg of wet weight | 13.3 ± 1.4 mg of protein/ mg of wet weight |
| IFM                      | 11.4 ± 0.6 mg of protein/ mg of wet weight | 9.8 ± 0.3 mg of protein/ mg of wet weight | 11.1 ± 0.7 mg of protein/ mg of wet weight |
| Uncoupled respiration: glutamate as complex I substrate |                      |                              |                                             |
| SSM                      | 201 ± 10 nAO/min of protein | 102 ± 5 nAO/min of protein | 25 ± 4 nAO/min of protein |
| IFM                      | 288 ± 16 nAO/min of protein | 172 ± 11 nAO/min of protein | 62 ± 8 nAO/min of protein |
| Respiration through cytochrome oxidase in SSM: TMPD-ascorbate as substrate |                      |                              |                                             |
| Max ADP                  | 477 ± 25 nAO/min of protein | 324 ± 20 nAO/min of protein | 436 ± 37 nAO/min of protein |
| Uncoupled                | 586 ± 32 nAO/min of protein | 338 ± 12 nAO/min of protein | 455 ± 41 nAO/min of protein |
| Respiration through cytochrome oxidase in IFM: TMPD-ascorbate as substrate |                      |                              |                                             |
| Max ADP                  | 800 ± 54 nAO/min of protein | 658 ± 35 nAO/min of protein | 813 ± 52 nAO/min of protein |
| Uncoupled                | 822 ± 59 nAO/min of protein | 651 ± 46 nAO/min of protein | 846 ± 55 nAO/min of protein |

\( ^a \) p < 0.05 versus non-ischemia hearts.  
\( ^b \) p < 0.05 versus 45 min ischemia alone.  
\( ^c \) Maximal rate of ADP-stimulated respiration using 2 mM ADP.  
\( ^d \) Dinitrophenol uncoupled respiration.

Polarographic cytochrome oxidase activity was measured using a Clark-type oxygen electrode at 30 °C (12). Cytochromes were quantified using the sumption in intact mitochondria was measured using a Clark-type electrode chamber (12). Ventricular pacing was discontinued during inhibitor administration. We previously found that cardiac function and mitochondrial oxidative metabolism were preserved during 60 min of perfusion as non-ischemic controls (n = 8) or 15 min of perfusion with rotenone (n = 8) or antimycin A (n = 3) or underwent no treatment (n = 10) followed by 45 min of global ischemia. Rotenone was used to inhibit complex I because of its high affinity for this complex and its irreversible inhibitory effect. Antimycin A is a high affinity inhibitor of complex III. Irreversible inhibition of complexes I and III was an advantage in the present study because reperfusion, and thus the need for recovery of oxidative metabolism, was not planned. Rotenone was administered as a bolus of 60 μM over 1 min immediately before ischemia. Antimycin A was infused at a final concentration of 400 μM over 1 min. The concentrations of inhibitors were calculated based upon the amount needed to yield a concentration of inhibitor in the estimated total tissue volume of the heart similar to the inhibitory concentrations used in the oxygen electrode chamber (12). Ventricular pacing was discontinued during inhibitor administration. We previously found that cardiac function and mitochondrial oxidative metabolism were preserved during 60 min of perfusion as time controls (18). Two populations of cardiac mitochondria were isolated as described previously (12, 16, 18). Oxygen consumption in intact mitochondria was measured using a Clark-type oxygen electrode at 30 °C (12). Cytochromes were quantified using the difference in oxygen uptake of mitochondrial-reduced minus ferricyanide-oxidized absorption spectra (12). Polarographic cytochrome oxidase activity was measured in deoxycholate-solubilized SSM in the presence of 32 μM cytochrome c and exogenous phospholipid supplied by liposomes of soybean asolectin as described previously (12). Complex III activity was measured as antimycin A sensitive decylubiquinol-cytochrome c reductase as described previously (28). Protein concentrations were measured using the biuret method with bovine serum albumin as standard (29).

Phospholipids were extracted from mitochondria, isolated from other lipid classes using silica gel columns (30), and separated into individual classes by normal phase HPLC as described previously (18, 31). Individual phospholipid and lysophospholipid peaks were identified by comparison of retention time to standards (18, 31). Organic phosphate was measured by the method of Bartlett adapted for use in a multiwell plate reader (18, 31). Organic phosphate analysis was not performed in two rotenone-treated hearts. In one heart, the standard curve in the regic phosphate assay did not meet quality control criteria. In the second heart, there was a technical failure of the total phospholipid phosphate measurement. Samples for lipid analysis were not available in two perfusion controls.

The rate of H2O2 production from SSM was measured by the oxidation of the fluorogenic indicator, Amplex Red, in the presence of horseradish peroxidase as described previously (32). Amplex Red reacts with H2O2 in the presence of horseradish peroxidase to generate a stable fluorescent product, resorufluor (excitation 530 nm, emission 585 nm). Fluorescence was measured using a microplate reader (1420 Victor2, PerkinElmer Life Sciences). SSM from non-ischemic hearts were incubated (0.1 mg protein/ml) at 30 °C in a reaction mixture containing cholexytreated 160 mM KCl, 10 mM potassium phosphate (pH 7.4), 1 mM EGTA, 50 μM/0.1units/ml of Amplex Red/horseradish peroxidase containing glutamate 10 mM, pyruvate (2.5 mM)/malate (2.5 mM), or succinate (10 mM) as substrates with or without either rotenone (2.4 μM), antimycin A (10 μM), or stigmatellin (6.6 μM) as inhibitors. H2O2 production was calculated as fluorescence minus background rates measured in the absence of SSM (pmol/mg protein). As expected, the addition of catalase (643 units/ml) decreased fluorescence by 85–90%, confirming that the increase in fluorescence occurred because of H2O2 production.

**RESULTS**

Rabbit Heart Model of Ischemia—The isolated, buffer-perfused rabbit heart was studied before ischemia (n = 8), following 45 min of ischemia (n = 10) or 45 min of ischemia with rotenone (n = 8) or antimycin A (n = 3) treatment immediately before ischemia. Before inhibitor treatment, all groups had similar developed pressure, positive and negative dP/dt, and coronary flow (data not shown). The bolus infusion of either inhibitor decreased developed pressure to less than 60% of the pressure before inhibitor administration without an increase in diastolic pressure or decrease in coronary flow (data not shown). Diastolic pressure following 45 min of ischemia was similar in the presence or absence of inhibitors (ischemia alone: 45 ± 4 mm Hg; n = 10; rotenone + ischemia: 41 ± 5 mm Hg; n = 8; antimycin A + ischemia: 38 ± 9 mm Hg; n = 3). Ischemia, with or without rotenone pretreatment, did not alter the protein yield of SSM or IFM compared with non-ischemic controls (Table I). Rotenone markedly inhibited glutamate oxidation, although inhibition was somewhat greater in SSM compared with IFM as expected (Table I). Antimycin A also did not alter mitochondrial protein yield (SSM 12.4 ± 0.6 mg of protein/gm heart, n = 3).

**Respiration through cytochrome oxidase in IFM: TMPD-ascorbate as substrate**

Max ADP                  | 800 ± 54 nAO/min of protein | 658 ± 35 nAO/min of protein | 813 ± 52 nAO/min of protein |
| Uncoupled                | 822 ± 59 nAO/min of protein | 651 ± 46 nAO/min of protein | 846 ± 55 nAO/min of protein |

Non-ischemia (n = 8)  
45 minutes ischemia (n = 10)  
45 minutes ischemia-rotenone treated (n = 8)

\( ^a \) p < 0.05 versus non-ischemia hearts.

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before ischemia with rotenone exhibited several morphological differences from mitochondria in untreated ischemic hearts (Fig. 1). The major alteration involved increased pallor of the inner compartment. A few of the mitochondria were swollen, signaled by an increase in size and a disrupted outer membrane. All of the mitochondria lacked matrix granules. In all other respects, the mitochondria, both subsarcolemmal and interfibrillar, were normal in appearance; most were of typical size, and cristae were unchanged in number or configuration.

Again as in our previous study, the mitochondrial pellets from untreated ischemic hearts consisted of a mixture of organelles, most of which were normal in appearance except for a pallid matrix (Fig. 2). Mitochondria isolated from rotenone-treated, ischemic hearts did not differ to any meaningful degree from their counterparts derived from untreated ischemic hearts. Although the majority of isolated SSM from such hearts had a typical morphology, a few showed disruption of the outer membrane, leading to the formation of "blowout" blebs of inner membrane. Some organelles contained a large vacuole that signaled by an increase in size and a disrupted outer membrane. All of the mitochondria lacked matrix granules. In all other respects, the mitochondria, both subsarcolemmal and interfibrillar, were normal in appearance; most were of typical size, and cristae were unchanged in number or configuration.

Next we asked if blockade of electron transport distal to the quinol oxidation (Qo) site of complex III with antimycin A also preserved oxidation through cytochrome oxidase. In contrast to rotenone, treatment immediately before ischemia with antimycin A failed to preserve the rate of TMPD-ascorbate oxidation through cytochrome oxidase in IFM, although a non-significant trend toward a decrease in oxidation was evident at the end of ischemia, also ameliorated by rotenone treatment (Table I). Pretreatment with Rotenone but Not with Antimycin A Preserves Oxidative Function through Cytochrome Oxidase—As described previously, the oxidation of TMPD-ascorbate, an electron donor to cytochrome oxidase via cytochrome c, decreased following 45 min of ischemia in SSM (12, 18). Following 45 min of ischemia in the presence of rotenone the rates of both ADP-stimulated and uncoupled respiration through cytochrome oxidase were higher than with ischemia alone (Table I). Rotenone treatment did not alter oxidative phosphorylation through cytochrome oxidase in IFM, although a non-significant pallor, with more mitochondria lacking matrix granules. Occasional swollen mitochondria are seen. Otherwise, the in situ mitochondria are fairly normal in appearance and in general are comparable with the appearance of mitochondria from untreated ischemic hearts (see A). Again, evidence of severe cytologic injury is not present.

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caused by this inhibitor. The decrease in cytochrome oxidase activity in SSM from ischemic hearts treated with antimycin A suggests that this inhibitor causes additional damage to cytochrome oxidase by a mechanism distinct from ischemia.

Content of Phospholipids in SSM and IFM from Rotenone-treated and Untreated Ischemic Hearts—Ischemia decreased the content of cardiolipin in SSM, whereas the cardiolipin content in IFM remained unaltered (18). The decrease in cardiolipin content is selective given that the contents of phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol are preserved during ischemia in SSM (18). Rotenone treatment preserved the content of cardiolipin in SSM following 45 min of ischemia (Fig. 3). Treatment with antimycin A before ischemia also preserved the content of cardiolipin in SSM (43 ± 7 nmol/mg, n = 3). Cardiolipin content (nmol/mg of protein) was unaltered by ischemia in IFM (non-ischemic 40 ± 5 (n = 6); ischemia alone 38 ± 2 (n = 10); rotenone-treated ischemia 49 ± 4 (n = 6); all p = not significant). Total lipid phosphate was measured in parallel samples. The recovery of phospholipids was excellent, as shown by the sum of the four

|                | SSM      | 45 min ischemia | 45 min ischemia-rotenone treated | 45 min ischemia-antimycin A treated |
|----------------|----------|-----------------|----------------------------------|------------------------------------|
| Non-ischemia   | 5335 ± 66| 5364 ± 213      | 5833 ± 273                       | 3834 ± 42*                         |
| (n = 3)        | (n = 3)  | (n = 3)         | (n = 2)                          |                                    |

*p < 0.05 versus all other groups by one-way analysis of variance. One antimycin A-treated heart did not have any mitochondria remaining for assay.

**Fig. 2.** Subsarcolemmal mitochondria (A and C) and interfibrillar mitochondria (B and D) isolated following 45 min of ischemia in the absence (A and B) and presence (C and D) of rotenone treatment (magnification ×7,400). A and B, following ischemia, SSM (A) and IFM (B) exhibit decreased matrix density and minor vacuolization of cristae compared with non-ischemic controls. Mitochondria are generally intact with occasional evidence of membranous fragments present in the background. C and D, most isolated SSM (C) exhibit a typical morphology similar in appearance to those from untreated hearts. Occasional IFM (D) display disruption of the outer membrane, swelling of the matrix, or a matrical pallor, with an apparent increase in the prevalence of these alterations compared with organelles from untreated, ischemic hearts.
individual phospholipids representing ~90% of total lipid phosphate (SSM, 88 ± 5%, n = 22; IFM, 88 ± 5%, n = 22), as described previously (18, 31).

Rotenone Treatment Preserves the Content of Cytochrome c in SSM during Ischemia—The content of cytochrome c decreases at 45 min of ischemia in SSM and in IFM. Rotenone treatment before ischemia blunted the decrease in cytochrome c content in SSM (Fig. 4). Treatment before ischemia with antimycin A also decreased the loss of cytochrome c from SSM (0.15 ± 0.03 nmol/mg, n = 3, ± S.E.) similar to the protection with rotenone. Inhibition of electron transport also preserved cytochrome c content in IFM (nmol/mg pr: non-ischemic, 0.27 ± 0.01 (n = 8); ischemia alone, 0.22 ± 0.01 (n = 10), (p < 0.05 versus non-ischemic; rotenone-treated ischemia, 0.25 ± 0.02 (n = 8), (p = not significant versus non-ischemia and ischemia alone)).

Rotenone Decreases Complex III-mediated Production of Reactive Oxygen Species in SSM—Complex III is the major site of production of reactive oxygen species during ischemia (2). The complex III inhibitor, antimycin A, augments oxyradical production during the oxidation of complex I and II substrates (34, 35). To address whether or not rotenone blockade of electron flow blunted the production of reactive oxygen species from complex III in isolated SSM, the production of H2O2 was studied in isolated rabbit heart SSM (Table III). Minimal H2O2 was produced by SSM in the absence of inhibitors of electron transport during the oxidation of pyruvate with malate as the complex I substrate (Table III). The presence of rotenone alone did not increase net H2O2 production. As expected, pyruvate oxidation in the presence of antimycin A led to a large increase in H2O2 production. Rotenone blockade markedly diminished the production of H2O2 by pyruvate oxidation in the presence of antimycin A. The blockade of electron entry into complex III by stigmatellin (36) also decreased antimycin A-induced H2O2 production. As expected, catalase decreased fluorescence. Similar results were obtained using glutamate as a second complex I substrate (data not shown). Thus, by limiting electron flow into complex III, rotenone blockade is effective in blunting even maximal complex III-driven oxyradical production during the oxidation of complex I substrates in intact SSM. These findings in rabbit heart SSM confirm our previous findings in rat heart mitochondria (27).

DISCUSSION

Myocardial ischemia leads to progressive damage to the electron transport chain. Ischemia decreases the content of the inner membrane phospholipid cardiolipin concomitant with a decrease in the content of cytochrome c and the rate of oxidative phosphorylation through cytochrome oxidase (18, 37). In the current study, blockade of the proximal electron transport chain with rotenone, a high affinity and irreversible inhibitor of complex I, markedly attenuated ischemic damage to cardiolipin, cytochrome c, and cytochrome oxidase. Even following the relatively prolonged ischemic period of 45 min, these targets of ischemic damage were protected. Thus, the mitochondrial electron transport chain itself is a source of the progressive damage that occurs to mitochondria during ischemia.

Mitochondria generate cytotoxic reactive oxygen species during ischemia (2, 19). The molecular targets of mitochondrial-derived oxidative damage during ischemia remain unknown. Cardiolipin is a phospholipid unique to mitochondria that is enriched in oxidatively sensitive acyl residues (26). During ischemia, the content of cardiolipin decreases (18, 38). The oxidation of cardiolipin forms peroxo groups that are unstable and rapidly decompose in the presence of metal ions (39, 40) or react to form phospholipid-protein adducts (41), leading to a decrease in cardiolipin content (18). In the present study, the inhibition of the electron transport chain by rotenone or antimycin A treatment immediately before ischemia preserves the content of cardiolipin, possibly reflecting a decrease in oxidative damage secondary to decreased generation of reactive oxygen species by mitochondria.

Cytochrome oxidase activity requires the presence of a cardiolipin-rich environment in the inner mitochondrial membrane (23, 24). During ischemia of the rabbit heart, the rate of oxidation through cytochrome oxidase decreases in concert with the decrement in cardiolipin content in SSM (18). Preservation of uncoupled electron transport through cytochrome oxidase (the most direct measurement of cytochrome oxidase activity in intact mitochondria) by rotenone probably reflects the preservation of cardiolipin content. The relationship between preservation of cardiolipin content and maintenance of the rate of oxidation through cytochrome oxidase is shown in Fig. 5.

Cardiolipin interacts with cytochrome c via non-ionic (42–44) and electrostatic (45) mechanisms to localize cytochrome c at the inner mitochondrial membrane (45). A decrease in cardiolipin content or the oxidative modification of cardiolipin in vitro diminishes the affinity of cytochrome c for the inner mitochondrial membrane (42–44). Cardiolipin depletion destabilizes cytochrome c from the inner membrane (45, 46), the first step leading to cytochrome c release by mitochondria (45). Following ischemia, the content of cytochrome c in SSM and IFM is correlated with the cardiolipin content in both rotenone-treated and untreated hearts (Fig. 6). It therefore is not surprising that preservation of cardiolipin content in SSM during...
Rotenone Prevents Ischemic Damage

TABLE III
Production of H$_2$O$_2$ (pmol/mg of protein/30 min) by isolated rabbit heart subsarcolemmal mitochondria oxidizing complex I substrate

| No inhibitor | ROT | AA | AA + ROT | AA + Stig | AA + Cata |
|--------------|-----|----|---------|----------|----------|
| 149 ± 20     | 120 ± 43 | 337 ± 66$^c$ | 106 ± 42$^b$ | 182 ± 73$^b$ | 82 ± 37$^b$ |

$^a$ p < 0.05 versus no inhibitor.

$^b$ p < 0.05 versus AA alone.

Complexes I and III are the major sites for the production of reactive oxygen species by the electron transport chain (34, 35). Rotenone blocks complex I near the binding site for ubiquinol, the electron acceptor from complex I (47). Block of complex I at this distal site in electron flow through the complex enhances the reduction of the NADH dehydrogenase site of complex I, the site of electron leak from the complex (47). Thus, rotenone blockade should enhance rather than decrease electron leak from complex I. In isolated mitochondria, rotenone alone does not enhance the net production of reactive oxygen species during the oxidation of complex I substrates (Table III) (27). The oxidants produced from the NADH dehydrogenase of complex I are probably directed into the mitochondrial matrix and are detoxified by matrix antioxidant enzymes (48) or may lead to oxidative stress in the matrix compartment. An increase in oxidative stress within the mitochondrial matrix may contribute to the subtle morphologic changes noted following ischemia in mitochondria from rotenone-treated hearts.

Complex III reduces molecular oxygen to form superoxide (O$_2^-$) (32, 35). Superoxide produced by complex III is released into the intermembrane space (49), favoring release from mitochondria (27, 50), shown for rabbit heart SSM (Table III). Antimycin A, an inhibitor of complex III that blocks distal to the Qo site of the complex, augments the net production of reactive oxygen species from complex III in isolated mitochondria (32, 35), including in cases where complex I substrates are oxidized (Table III). In contrast, stigmatellin, an inhibitor of complex III that blocks the entry of electrons into complex III at the Qo site, mitigates the production of reactive oxygen species (Table III). Rotenone markedly attenuates the antimycin A-stimulated production of reactive oxygen species during the oxidation of complex I substrates (27) (Table III). In vitro, in bovine submitochondrial particles electron flow into complex III in the presence of antimycin A blockade appears to deplete cardiolipin (51) and also favors the loss of cytochrome c from liver mitochondria (45).

Rotenone treatment of isolated myocytes decreased the production of reactive oxygen species during simulated ischemia (2). The observations of decreased oxidant production by isolated mitochondria in the presence of stigmatellin agree with the observation in cardiac myocytes that myxothiazol, an inhibitor of complex III that also blocks electron entry into the complex (32, 35), decreased the production of reactive oxygen species in cardiomyocytes during simulated ischemia (2). The decrease in net production of reactive oxygen species in mitochondria and myocytes suggests that the major site of the production of reactive oxygen species that are released from mitochondria during myocardial ischemia is distal to the quinol reduction site of complex I, the site of rotenone block, and is likely the Qo site of complex III.

The use of inhibitors that block electron flow distal to complex I was considered to further localize the sites in the electron transport chain that contribute to mitochondrial damage during ischemia. The next site to consider is the Qo site of complex III and the use of stigmatellin or myxothiazol to block this site. Unfortunately, stigmatellin at higher concentrations also inhibits complex I at the rotenone site (52). During bolus...
administration of the inhibitor to the isolated heart in the minute before ischemia, administration of stigmatellin will also block complex I, making interpretation of experimental results difficult. Myxothiazol also inhibits complex I at the rotenone site (52, 53). Antimycin A blockade of complex III distal to the Qo site immediately before ischemia preserved the contents of cardiolipin and cytochrome c. Thus, inhibition of electron transport at either the rotenone binding site in complex II or the antimycin A binding site in complex III markedly attenuates the decrease in cardiolipin and cytochrome c contents during ischemia. These results suggest that the potential site(s) in the electron transport chain that lead to the depletion of cardiolipin and cytochrome c during ischemia are probably distinct from the Qo site of complex III that favors the release of reactive oxygen species from mitochondria into the remainder of the cell.

In contrast to rotenone, treatment immediately before ischemia with antimycin A failed to preserve the rate of oxidative phosphorylation or uncoupled respiration with TMPD-ascorbate as substrate in SSM following ischemia. The disparate results with antimycin A treatment between oxidation through cytochrome oxidase in intact mitochondria and preservation of the contents of cardiolipin and cytochrome c strongly suggest that treatment with antimycin A damaged cytochrome oxidase independent of cardiolipin depletion. This proposal was confirmed by the finding of a decrease in polargraphic cytochrome oxidase activity in solubilized SSM in the presence of exogenous phospholipid only in antimycin A-treated hearts (Table II). Thus, antimycin A treatment before ischemia damages cytochrome oxidase by a mechanism distinct from ischemia alone, perhaps damage to subunit peptides of the complex, that leads to decreased respiration through cytochrome oxidase despite the preserved contents of cardiolipin and cytochrome c in SSM from antimycin A hearts.

The present study provides evidence that ischemic damage to mitochondria is mediated at least in part by the mitochondria themselves. The electron transport chain participates in the sequence of events that deplete cardiolipin during ischemia, leading in turn to a decrease in cytochrome c content and decreased oxidation through cytochrome oxidase. Ischemic damage to mitochondria leads to the production of reactive oxygen species (2), to the onset of permeability transition (54), and to the release of cytochrome c (54) during reperfusion. Cytochrome c release activates cell death programs (54, 55), and to the release of cytochrome c (54) during reperfusion. Cytochrome c release activates cell death programs (54, 55), and to the release of cytochrome c (54) during reperfusion. Cytochrome c release activates cell death programs (54, 55), and to the release of cytochrome c (54) during reperfusion. Cytochrome c release activates cell death programs (54, 55), and to the release of cytochrome c (54) during reperfusion. Cytochrome c release activates cell death programs (54, 55), and to the release of cytochrome c (54) during reperfusion. Cytochrome c release activates cell death programs (54, 55), and to the release of cytochrome c (54) during reperfusion.
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