In Vitro Study on Contribution of Oxidative Metabolism of Isolated Rabbit Heart Mitochondria to Myocardial Reperfusion Injury

Hajime Otani, Hidehiko Tanaka, Takeo Inoue, Masao Umemoto, Kazuo Omoto, Kazuho Tanaka, Tadashi Sato, Tsutomu Osaka, Atoh Masuda, Akira Nonoyama, and Terumasa Kagawa

SUMMARY. The present study was performed to clarify oxygen-induced damage following myocardial reperfusion, using three mitochondrial preparations from isolated rabbit hearts (non-ischemic hearts and those subjected to 40 and 90 minutes of normothermic global ischemia). The viability of mitochondria was evaluated by adenosine triphosphate generation. The extent of mitochondrial injury produced by reactive oxygen metabolism was assessed by the intensity of hydroxyl radical signal detected with electron spin resonance spectroscopy and the reduction of coenzyme Q10 level. The greatest oxygen-induced injury was observed in 40-minute ischemic mitochondria exposed to pure oxygen. The use of superoxide dismutase and catalase satisfactorily prevented the oxygen-induced injury. Moreover, the net adenosine triphosphate generation of the 40-minute ischemic mitochondria was comparable to that of the nonischemic mitochondria without the enzymes. These results suggest that reperfusion of the ischemic myocardium with viable mitochondria is deleterious, because mitochondria are susceptible to injury resulting from oxidative metabolism, and that the use of superoxide dismutase, together with catalase, is beneficial for the restoration of cardiac function after ischemia. (Circ Res 55: 168-175, 1984)

REVASCULARIZATION, both surgical and nonsurgical, after coronary occlusion, is an established therapeutic procedure for patients with acute myocardial infarction. However, the contribution of successful revascularization to myocardial salvage is still controversial. There is no doubt that irreversible myocardial damage will develop unless reperfusion is performed within several hours. Although some authors suggest that reperfusion should be carried out even after extended periods of coronary occlusion (Maroko et al., 1972; Ginks et al., 1972; Puri, 1975; Reimer et al., 1977), others have drawn attention to the possible detrimental effects of reperfusion after coronary occlusion (Maroko et al., 1975; Sugiyama et al., 1980a; Bulkley and Hutchins, 1977; Smith et al., 1978).

Despite the evidence of functional, metabolic, and structural abnormalities during reperfusion, the precise mechanisms of reperfusion injury remain obscure because reperfusion exhibits multifactorial effects on the ischemic myocardium. Thus, it is necessary to examine the characteristic events occurring in the myocardium and evaluate the factors involved in the recovery of myocardial function during the post-perfusion period.

Reperfusion results in abrupt delivery of oxygen and substrates to the myocardium, which the mitochondria utilize principally for energy production; thus, the initiation of mitochondrial respiration is considered a characteristic phenomenon of reperfusion. On the other hand, mitochondria are regarded as being the most susceptible to ischemia among the cellular components, and it appears that reperfusion itself has deleterious effects on mitochondrial function (Kane et al., 1975; Sugiyama et al., 1980b). Since the recovery of myocardial contraction after ischemia is ultimately dependent on the available energy supply, the inability of the mitochondria to produce sufficient energy would soon result in myocardial dysfunction.

Therefore, we investigated the relationship between mitochondrial oxidative metabolism and cardiac reperfusion injury, using in vitro models. The efficacy of activated oxygen scavengers, superoxide dismutase (SOD), and catalase, in protecting against such injury was also examined.

Methods

Rabbits of both sexes, weighing 2-3 kg, were lightly anesthetized with sodium pentobarbital injected intravenously, and their hearts were rapidly excised. Three normothermic mitochondrial preparations were studied: nonischemic, and those obtained from hearts subjected to 40 and 90 minutes of global ischemia. For the isolation of rabbit heart mitochondria (Chance and Haghara, 1963), the ventricle was minced in ice-
Determination of ATP Generation

Incubation of Mitochondria

State 4 respiration was initiated by incubation of 1.0 ml of the mitochondrial suspension in a reaction medium (0.25 M sucrose, 5 mM Tris, 20 mM inorganic phosphate, 0.25 mM MgCl₂, 0.25 mM EDTA, and 5 mM succinate as a substrate at pH 7.4) which had been equilibrated with oxygen, room air, and nitrogen in test tubes at 30°C. Transition from state 4 to state 3 respiration was induced by the addition of 0.1 ml of 0.5 mM adenosine diphosphate (ADP). The same amounts of ADP and 0.1 ml of 1.0 mM succinate were added 15 minutes after the start of state 3 respiration. ESR spectra were recorded with a JEOL JES FE-1X spectrometer at 10-minute intervals after the start of incubation in order to determine the mitochondrial coenzyme Q₁₀ content and ATP generation.

Identification of Hydroxyl Radical

Since many investigators have failed to detect short-lived oxygen radicals directly, electron spin resonance (ESR) spectroscopy, the ESR-spin trapping technique, using 5,5'-dimethyl-1-pyrroline-1-oxide (DMPO, Sigma) as a spin-trapping agent, was employed to detect oxy-radicals being generated in respiring mitochondria. In this experiment, 0.5-ml aliquots of mitochondria (8 mg of protein/ml) were incubated in 4.5 ml of the above-mentioned reaction medium to which was added 0.1 ml of 10 mM succinate aerated with nitrogen (Fig. 1A). Since this spectrum consisted of a 1:2:2:1 quartet with hyperfine splittings of aₙ = aₚ = 15.3 G, it was identified as a DMPO-OH adduct signal (Harbour et al., 1974). The same spectra were obtained in the nonischemic and 90-minute ischemic mitochondria though the signal height was diminished (Fig. 1, B and C).

SOD and Catalase

Shortly before the incubation, 1 μM bovine liver SOD (specific activity of 3000 U/mg protein) and 0.2 μM bovine liver catalase (specific activity of 2000 U/mg protein) were added to the reaction medium. Both agents were obtained from Sigma Chemical Co.

Results

Observation of Hydroxyl Radical

A distinct ESR spectrum appeared in state 4 respiration of the 40-minute ischemic mitochondria aerated with oxygen (Fig. 1A). Since this spectrum consisted of a 1:2:2:1 quartet with hyperfine splittings of aₙ = aₚ = 15.3 G, it was identified as a DMPO-OH adduct signal (Harbour et al., 1974). The same spectra were obtained in the nonischemic and 90-minute ischemic mitochondria though the signal height was diminished (Fig. 1, B and C).

The signal height of the DMPO-OH adduct was compared among the mitochondrial groups during state 4 respiration aerated with oxygen. The height of the low field line in the DMPO-OH adduct signal was monitored, and relative intensity of the ESR signal of nonischemic and 90-minute ischemic mito-

Determination of Coenzyme Q₁₀

For determination of the mitochondrial coenzyme Q₁₀ content (Ikenoya et al., 1981), 0.5 ml of mitochondrial suspension was placed into a test tube containing 8 ml of ethanol/n-hexane (3:5 by volume). The tube was shaken to extract the coenzyme Q₁₀, which was dried under a stream of nitrogen and analyzed by high performance liquid chromatography.

Determination of ATP Generation

State 3 respiration was terminated by placing the mitochondrial suspension (0.5 ml) in a test tube containing 2.5 ml of 0.6 N perchloric acid (PCA). After centrifugation of the PCA extract, the supernatant was adjusted to pH 7.0 and was used for determining ATP according to the enzymatic procedure (Bücher, 1947) using a test combination (Boehringer Manheim Company).

Statistical Analysis

Student's t-test for paired data was used for statistical analysis, and results were expressed as mean ± SD.

Figure 1. ESR spectra of the DMPO adducts formed by the trapping of hydroxyl (OH) radical obtained from 40-minute ischemic mitochondria (panel A), nonischemic mitochondria (panel B), and 90-minute ischemic mitochondria (panel C) with state 4 respiration aerated with oxygen. Arrows represent hyperfine splittings of DMPO-OH adduct. Each reaction mixture contains 0.8 mg of mitochondrial protein/ml. ESR conditions are microwave power 20 mW, modulation width 2.0 gauss, time constant 1.0 second, and scan time 8 minutes.
Mitochondria were calculated in relation to the mean signal height of the 40-minute ischemic group. Relative intensity of ESR signal in the 40-minute ischemic group was significantly greater than those of nonischemic and 90-minute ischemic groups (Table 1). The hydroxyl radical signal during state 3 respiration of the 40-minute ischemic mitochondria was not as high as the additional signal, which is independent of the hydroxyl radical and likely to be associated with reactive oxygen intermediates, but could not be identified in these experiments (Fig. 2A).

### TABLE 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Relative intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonischemia (n = 8)</td>
<td>0.77 ± 0.08</td>
</tr>
<tr>
<td>40-Min ischemia (n = 8)</td>
<td>1.00 ± 0.17*</td>
</tr>
<tr>
<td>90-Min ischemia (n = 8)</td>
<td>0.56 ± 0.10</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. * Significant difference from nonischemic group; P < 0.01. † Significant difference from 90-minute ischemic group; P < 0.001.

During state 4 respiration, room air had no significant effect on the hydroxyl radical signal of the 40-minute ischemic mitochondria (Fig. 2B), whereas exposure to nitrogen completely inhibited the ESR signal (Fig. 2C). No DMPO adducts were formed during state 1 respiration, even when 40-minute ischemic mitochondria were continuously exposed to oxygen (Fig. 2D).

The incubation of 40-minute ischemic mitochondria treated with SOD and catalase under oxygen exposure markedly suppressed the appearance of hydroxyl radical signal, during state 4 (Fig. 3A) and state 3 respiration (Fig. 3B). It is known that hydroxyl radical is generated dramatically in the H$_2$O$_2$ generation system in the presence of a catalytic amount of iron. Therefore, in order to examine whether the buffers used in this study contained a sufficient amount of iron to influence the intensity of DMPO-OH adduct signal, we conducted ESR measurements with the buffer containing an excess amount of H$_2$O$_2$ (50 mM). Since DMPO-OH adduct signal was not detected, we may assume that the buffer used in our experiment did not contain a sufficient amount of iron to change the ESR signal significantly.

In addition, the intensity of the DMPO-OH adduct signal in ESR measurement of the buffer treated with Chelex 100 resin was nearly identical to that of the buffer untreated with the same resin.

### Level of Coenzyme Q$_{10}$ Content in Mitochondria

As shown in Table 2, the coenzyme Q$_{10}$ content in mitochondria during state 3 respiration decreased in every gas phase among the three groups. Aeration with oxygen of nonischemic mitochondria resulted in a significant reduction in the coenzyme Q$_{10}$ content, not only compared with the control value, but also with aeration using room air.

Coenzyme Q$_{10}$ content of the 40-minute ischemic group, whose control value was 77% that of the nonischemic group, was reduced by 37% of the control value after 30-minute incubation in oxygen. A significant difference was observed between the oxygen and room air gas phases as early as 10 minutes after incubation. Levels of coenzyme Q$_{10}$

![Figure 2. ESR spectra of the DMPO adducts observed in 40-minute ischemic mitochondria generated during state 3 respiration with oxygen (panel A), during state 4 respiration with either room air (panel B), or nitrogen (panel C), or during state 1 respiration with oxygen (panel D). Each reaction mixture contains 0.8 mg of mitochondrial protein/ml. ESR conditions are the same as shown in Figure 1.](http://circres.ahajournals.org/)

![Figure 3. ESR spectra of the DMPO adducts obtained from 40-minute ischemic mitochondria treated with SOD and catalase under oxygen exposure in state 4 respiration (panel A) and state 3 respiration (panel B). Arrows indicate the appearance of the hydroxyl radical signal. The mitochondria used here are the same as those in Figure 1A. Reaction mixtures contain 1 μM SOD and 0.2 μM catalase. ESR conditions are the same as shown in Figure 1.](http://circres.ahajournals.org/)
content during aeration with nitrogen showed mid-values between aeration with room air and oxygen. The reduction of coenzyme Q_{10} content in the 90-minute ischemic group, whose control value was 44% that of the nonischemic group, was inhibited considerably in every gas phase compared with the other two groups. Whereas aeration with oxygen and nitrogen showed significant reductions in coenzyme Q_{10} content from the control values 30 minutes after incubation, aeration with room air did not. In this group, the differences in coenzyme Q_{10} content among the three gas phases were not significant.

In the 40-minute ischemic mitochondria, changes in the level of coenzyme Q_{10} during state 4 respiration were similar to those during state 3. In contrast, no changes in any of the gas phases were seen during state 1 respiration (Table 3).

Table 4 shows the effect of SOD and catalase on the change in coenzyme Q_{10} content of mitochondria during state 3 respiration. A significant reduction in coenzyme Q_{10} content was observed after 30 minutes of incubation with oxygen, but not with room air. Thus, the administration of the enzymes to 40-minute ischemic mitochondria, which exhibited the largest hydroxyl radical generator, satisfactorily preserved coenzyme Q_{10} under aerobic conditions, but had no effect on anaerobically respiring mitochondria.

### Mitochondrial ATP Generation

The time courses of mitochondrial ATP generation during state 3 respiration are plotted in Figure 4. A significant difference in ATP generation between room air and oxygen exposure, was seen 20–30

### Table 2

**Time Course of Change in the Level of Coenzyme Q_{10} Content in Mitochondria with State 3 Respiration**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonischemia (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen exposure</td>
<td>3.80 ± 0.35</td>
<td>2.97 ± 0.27§</td>
<td>2.60 ± 0.21†</td>
<td>2.45 ± 0.27§</td>
</tr>
<tr>
<td>Room air exposure</td>
<td>3.39 ± 0.30</td>
<td>3.07 ± 0.25$</td>
<td>2.93 ± 0.25$</td>
<td>2.93 ± 0.25$</td>
</tr>
<tr>
<td>Nitrogen exposure</td>
<td>3.23 ± 0.31‡</td>
<td>2.97 ± 0.32‡</td>
<td>2.59 ± 0.24§</td>
<td>2.59 ± 0.24§</td>
</tr>
<tr>
<td>40-Min ischemia (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen exposure</td>
<td>2.91 ± 0.27</td>
<td>2.25 ± 0.22§§</td>
<td>2.03 ± 0.20‡§</td>
<td>1.84 ± 0.24‡§</td>
</tr>
<tr>
<td>Room air exposure</td>
<td>2.66 ± 0.19</td>
<td>2.49 ± 0.19*</td>
<td>2.39 ± 0.23‡</td>
<td>2.39 ± 0.23‡</td>
</tr>
<tr>
<td>Nitrogen exposure</td>
<td>2.34 ± 0.26‡</td>
<td>2.14 ± 0.22‡§</td>
<td>1.99 ± 0.17‡</td>
<td></td>
</tr>
<tr>
<td>90-Min ischemia (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen exposure</td>
<td>1.69 ± 0.21</td>
<td>1.46 ± 0.14</td>
<td>1.37 ± 0.17*</td>
<td>1.29 ± 0.19†</td>
</tr>
<tr>
<td>Room air exposure</td>
<td>1.64 ± 0.21</td>
<td>1.49 ± 0.24</td>
<td>1.42 ± 0.20</td>
<td>1.42 ± 0.20</td>
</tr>
<tr>
<td>Nitrogen exposure</td>
<td>1.63 ± 0.20</td>
<td>1.54 ± 0.17</td>
<td>1.39 ± 0.14*</td>
<td>1.39 ± 0.14*</td>
</tr>
</tbody>
</table>

Values (nmol/mg protein) are expressed as mean ± SD.

*†, § Significant difference from control; P < 0.02, P < 0.01, P < 0.001, respectively.

§, ‡ Significant difference from room air at the same incubation period; P < 0.02, P < 0.01, respectively.

### Table 3

**Time Course of Change in the Level of Coenzyme Q_{10} Content in 40-Min Ischemic Mitochondria with States 4 and 1 Respiration**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>State 4 respiration (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen exposure</td>
<td>3.02 ± 0.35</td>
<td>2.42 ± 0.16†§</td>
<td>2.27 ± 0.10§§</td>
<td>1.98 ± 0.19§§</td>
</tr>
<tr>
<td>Room air exposure</td>
<td>2.89 ± 0.31</td>
<td>2.78 ± 0.27</td>
<td>2.56 ± 0.18*</td>
<td>2.56 ± 0.18*</td>
</tr>
<tr>
<td>Nitrogen exposure</td>
<td>2.75 ± 0.24</td>
<td>2.55 ± 0.28</td>
<td>2.33 ± 0.27†</td>
<td>2.33 ± 0.27†</td>
</tr>
<tr>
<td>State 1 respiration (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen exposure</td>
<td>2.87 ± 0.16</td>
<td>2.76 ± 0.26</td>
<td>2.74 ± 0.31</td>
<td>2.75 ± 0.21</td>
</tr>
<tr>
<td>Room air exposure</td>
<td>2.76 ± 0.31</td>
<td>2.79 ± 0.33</td>
<td>2.78 ± 0.27</td>
<td>2.78 ± 0.27</td>
</tr>
<tr>
<td>Nitrogen exposure</td>
<td>2.85 ± 0.28</td>
<td>2.84 ± 0.20</td>
<td>2.84 ± 0.23</td>
<td>2.84 ± 0.23</td>
</tr>
</tbody>
</table>

Values (nmol/mg protein) are expressed as mean ± SD.

*†, ‡ Significant difference from control; P < 0.02, P < 0.01, P < 0.001, respectively.

§, ‡ Significant difference from room air at the same incubation period; P < 0.01, P < 0.001, respectively.
TABLE 4
Effect of SOD and Catalase on Change in the Level of Coenzyme Q10 Content in 40-Min Ischemic Mitochondria with State 3 Respiration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>10 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SOD + catalase administered (n = 6)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen exposure</td>
<td>2.76 ± 0.20</td>
<td>2.64 ± 0.27</td>
<td>2.49 ± 0.26*</td>
<td></td>
</tr>
<tr>
<td>Room air exposure</td>
<td>2.91 ± 0.22</td>
<td>2.90 ± 0.24</td>
<td>2.78 ± 0.19</td>
<td>2.68 ± 0.17</td>
</tr>
<tr>
<td>Nitrogen exposure</td>
<td>2.40 ± 0.22†§</td>
<td>2.13 ± 0.25§</td>
<td>2.05 ± 0.23</td>
<td></td>
</tr>
</tbody>
</table>

Values (nmol/mg protein) are expressed as mean ± SD.

* Significant difference from control; P < 0.02, P < 0.01, P < 0.001, respectively.
†, ‡ Significant difference from room air at the same incubation period; P < 0.01, P < 0.001, respectively.
§, || Significant difference from oxygen exposure.

minutes after incubation in the nonischemic mitochondria. ATP generation during nitrogen exposure was less than one-third of that observed during room air exposure, probably due to a slight oxygen contamination.

Similar time courses were observed in the 40-minute ischemic group. However, the difference in ATP generation between these two aerobic conditions was more obvious 20 and 30 minutes after incubation than in the nonischemic group. Little ATP generation was seen in the 90-minute ischemic group under aerobic condition.

The administration of SOD and catalase, enhanced ATP generation in 40-minute ischemic mitochondria under aerobic conditions (Fig. 5). The impairment of oxidative phosphorylation seen with oxygen was not observed in this group when the enzymes were added. Moreover, the net ATP generation under aerobic conditions with the enzymes was comparable to that in the nonischemic group aerated with room air. However, the enzymes had no effect on ATP generation of mitochondria aerated with nitrogen.

Discussion

The assumption that myocardial reperfusion causes unfavorable results has increasingly pervaded the field of cardiac research. Despite the accumulation of evidence concerning reperfusion injury, no exact mechanisms have yet been confirmed. The harmful effect of reoxygenation on the myocardium has been discussed from the aspect of contractile abnormality (Bing et al., 1971, 1976), ultrastructural damage (Feuvray and Leiris, 1975; Hearse et al., 1975, 1978; Ganote and Kaltenbach, 1979), and enzyme release (Ganote et al., 1976, Hearse et al., 1978; Ganote and Kaltenbach, 1979).

Our interest in reperfusion injury has been focused on the deterioration of the mitochondria. In our study, mitochondria whose respiration had been terminated as a result of oxygen and substrate deprivation during ischemia and/or preparation of mitochondria at low temperature showed an apparent impairment of phosphorylative activity in the presence of high concentrations of oxygen. Therefore, oxygen itself appears to be a major determinant of reperfusion injury for the following reasons.
zyme Q10, because of its potent antioxidant activity, Sugiyama et al., 1980a). Thus, the endogenous coenzyme Q0 might function as an antioxidant, and recent studies have stressed its antioxidative action (Takayanagi et al., 1980; Sledge et al., 1965; Bidlack and Tappel, 1973), which cleavage leads to the loss of homeostasis and specific protein constituents, and nucleotides. Above all, phospholipids containing abundant unsaturated free fatty acids are easily peroxidized. Since phospholipids are the principal element of cytoplasmic structures and biomembranes, their peroxidative cleavage leads to the loss of homeostasis and specific activity of cellular organelas (Hunter et al., 1963; Sledge et al., 1965; Bidlack and Tappel, 1973), which causes irreversible cell death unless an adequate amount of antioxidants terminates the propagation of peroxidation.

An earlier report (Mellors and Tappel, 1966) suggested that reduced coenzyme Q10 might function as an antioxidant, and recent studies have stressed its antioxidative action (Takayanagi et al., 1980; Sugiyama et al., 1980a). Thus, the endogenous coenzyme Q10, because of its potent antioxidant activity, is reduced by respiratory-dependent lipid peroxidation.

The beneficial effect of coenzyme Q10 on mitochondria clearly involves more than the antioxidative action; it might be related more closely to structural effects (Kobayashi et al., 1980) and activation of the respiratory enzymes of the electron transfer system (Szarkowska, 1966; Norling et al., 1974). Therefore, the depletion of coenzyme Q10 triggered by lipid peroxidation might affect mitochondrial respiratory function and the integrity of the membrane structure, and eventually impair phosphorylative activity; it is possible that activated oxygen directly inhibits mitochondrial respiratory function. Therefore, the parallel observed in the present study between the reduction of coenzyme Q10, along with the formation of hydroxyl radical and the reduction in ATP generation, seems to be of importance.

At present, a persuasive discussion of the mechanism of the coenzyme Q10 reduction in the mitochondria under nitrogen atmosphere, in which no significant generation of oxygen radicals was observed, is impossible. However, the present study, as well as other recent works demonstrating a considerable decrease in coenzyme Q10 in myocardium and/or mitochondria due to regional and global myocardial ischemia (Yamasawa et al., 1980; Chiba, 1982), seems to support the following speculation: State 3 and state 4 respiration of the mitochondria in a nitrogen atmosphere in the presence of available substrates is identical to the environment of ischemic mitochondrial. Thus, an excessive oxidation-reduction state of the respiratory chain of mitochondria may have caused the coenzyme Q10 reduction by an unknown mechanism.

High oxygen tension was demonstrated to increase H2O2 release of mitochondria (Boveris and Chance, 1973; Turrens et al., 1982). Our study also suggested that oxygen-induced mitochondrial injury was caused by the enhancement of mitochondrial respiration during excessive oxygen exposure. In addition, the fact that formation of hydroxyl radical and reduction of coenzyme Q10 was not observed in state 1 respiration, in which oxygen consumption is negligible due to the absence of exogenous substrate despite the presence of available oxygen, indicates that oxygen-induced mitochondrial injury does not result from only a high oxygen environment.

Moderately damaged mitochondria are known to consume more oxygen than do intact mitochondria during state 4 respiration, in which oxygen consumption is uncoupled with oxidative phosphorylation and may be attributable to the generation of activated oxygen (Loschen et al., 1971; Boveris and Chance, 1973). On the other hand, the myocardium contains little SOD or catalase in comparison with other principal tissue (Hein et al., 1975; Thayer, 1977; Doroshow et al., 1980), and the antioxidant ability would be further reduced by ischemia (Majewska et al., 1978; Guarnieri et al., 1980). This may
explain why the 40-minute ischemic mitochondria, which appeared to be moderately damaged, were not significantly affected by oxygen toxicity than the less-damaged nonischemic and severely damaged 90-minute ischemic mitochondria. The border zone of the infarct area, consisting of salvageable myocardium, must also be subjected to oxygen-induced injury. The deleterious effect of high concentrations of intramyocardial calcium and of the use of inotropic drugs in the post-perfusion period may be involved in oxygen-induced injury due to the enhancement of oxygen consumption.

Lack of the enzymatic defense of the heart mitochondria against oxygen toxicity led us to use SOD and catalase. These enzymes successfully inhibited the formation of hydroxyl radical, the reduction of coenzyme Q₁₀, and the impairment of oxidative phosphorylation. However, administration of SOD or catalase alone is insufficient to prevent oxygen-induced injury (Fong et al., 1973; Nohl et al., 1981), because each enzyme is inactivated in the presence of superoxide anion and hydrogen peroxide (Bray and Cockle, 1974; Kono and Fridovich, 1982). Combined administration of both enzymes is considered to be an effective and feasible preventive measure against oxygen-induced mitochondrial injury. The enzymatic decomposition of superoxide anion and hydrogen peroxide fundamentally removes the sources of peroxidation, while antioxidants such as coenzyme Q₁₀, α-tocopherol, and ascorbate indirectly remove reactive oxygen metabolites through the peroxidative reaction.

We express our gratitude to Eisai Company for their assistance in the measurement of coenzyme Q₁₀, as well as to Dr. Eiji Kimoto, Department of Chemistry, Faculty of Science, Fukui University, Fukuka, Japan, and Dr. Fukumi Morishige, Tachiarai Hospital, Fukuka, Japan, for their helpful advice.

This work was supported by a grant-in-aid (57480284) for scientific research from the Ministry of Education, Science, and Culture of Japan.

Address for reprints: Professor T. Kagawa, Department of Thoracic and Cardiovascular Surgery, Kansai Medical University, Moriguchi, Osaka, 570 Japan.

Received October 31, 1983; accepted for publication April 20, 1984.

References


Bidlack WR, Tappel AL (1973) Damage to microsomal membrane by lipid peroxidation. Lipids 8: 177–182


Feuvray D, Leiris J (1975) Ultrastructural modifications induced by reoxygenation in the anoxic isolated rat heart. J Mol Cell Cardiol 7: 307–314


Hearse DJ, Humphrey SM, Nayar WQ, Slade A, Border D (1975) Ultrastructural damage associated with reoxygenation of the anoxic myocardium. J Mol Cell Cardiol 7: 315–324


Kane JJ, Murphy ML, Bissett JK, Soyna N, Doherty JE, Straub KD (1975) Mitochondrial function, oxygen extraction, epicardial S-T segment changes and intratidal digoxin distribution after reperfusion of ischemic myocardium. Am J Cardiol 36: 218–224


Otani et al. /Oxygen-Induced Myocardial Reperfusion Injury

Oxidation of myocardial reperfusion by coenzyme Q10. Arch Biochem Biophys 217: 411–421
Vladimirov YA, Ofman VI, Suslova TB, Cheremisina ZP (1980) NADH- and NADPH-dependent lipid peroxidation in bovine heart mito-

INDEX TERMS: Reperfusion injury • Mitochondria • Coenzyme Q10 • Oxygen-induced injury • Superoxide dismutase

Vladimirov YA, Ofman VI, Suslova TB, Cheremisina ZP (1980) NADH- and NADPH-dependent lipid peroxidation in bovine heart mito-

INDEX TERMS: Reperfusion injury • Mitochondria • Coenzyme Q10 • Oxygen-induced injury • Superoxide dismutase
In vitro study on contribution of oxidative metabolism of isolated rabbit heart mitochondria to myocardial reperfusion injury.

H Otani, H Tanaka, T Inoue, M Umemoto, K Omoto, K Tanaka, T Sato, T Osako, A Masuda and A Nonoyama

*Circ Res.* 1984;55:168-175
doi: 10.1161/01.RES.55.2.168

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/55/2/168

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at: http://circres.ahajournals.org/subscriptions/