Biochimica et Biophysica Acta 890 (1987) 82–88
Elsevier

BBA 42158

Peroxidative injury of the mitochondrial respiratory chain during reperfusion of hypothermic rat liver

Toshirou Nishida [a], Hideo Shibata [b], Masato Koseki [a], Kazuyasu Nakao [a], Yasunaru Kawashima [a], Yukuo Yoshida [b] and Kunio Tagawa [b]

[a] *First Department of Surgery, Osaka University Medical School, 1-1-50 Fukushima, Fukushima-ku, Osaka 553, and* [b] *Department of Physiological Chemistry, Osaka University Medical School, 4-3-57 Nakanoshima, Kita-ku, Osaka 530 (Japan)*

(Received 23 June 1986)

Key words: Ischemia; Oxidative phosphorylation; Lipid peroxidation; Electron transport; (Rat liver mitochondria)

Mitochondrial dysfunction in ischemic liver has been demonstrated to be due to decrease in the intramitochondrial level of ATP and the subsequent disruption of the proton barrier of the inner membrane (Watanabe, F., Hashimoto, T. and Tagawa, K. (1985) J. Biochem. 97, 1229–1234). In this study, another injury process, impairment of the electron-transfer system, which occurred during reoxygenation of ischemic liver, was studied during reperfusion of cold preserved liver and during cold incubation of isolated rat-liver mitochondria. The sites of the respirtory chain that were sensitive to peroxidative damage were ubiquinone-cytochrome *c* oxidoreductase and NADH-ubiquinone oxidoreductase. These enzymic activities decreased with increase in lipid peroxidation. Incubation of submitochondrial particles with *t*-butyl hydroperoxide or with an NADPH-dependent peroxidation system decreased the enzymic activities of the electron-transport system. These data strongly suggested that lipid peroxidation during reoxygenation of ischemic liver impaired the electron-transfer system. Thus, mitochondria of ischemic liver suffer from two different types of injury: increase in proton permeability during anoxia, and decrease in enzymic activities of the electron-transport system during reoxygenation.

Introduction

Prolonged ischemia causes functional and structural damage of cells, and finally leads to cell death. The biochemical mechanism of cell death is still not fully understood, but it has been widely accepted that irreversible damage in ischemia is due to loss of the oxidative phosphorylation capacity of mitochondria [1–3]. Previously, we demonstrated that mitochondrial dysfunction during ischemia was caused by increased proton permeability through the inner mitochondrial membrane [4], and that this increase in proton permeability was associated with decrease of the intramitochondrial ATP level, which triggered release of Ca^{2+} from mitochondria [5].

Another biochemical mechanism of ischemic damage has been reported by several workers [6–8]. This is called 'reperfusion injury', and is the damage caused by peroxidation of various cellular components during reperfusion of ischemic organs. This peroxidation is known to occur on intracellular production of activated oxygen [9], and to

Abbreviations: DCIP, 2,6-dichloro-indophenol; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

Correspondence: K. Tagawa, Department of Physiological Chemistry, Osaka University Medical School, 4-3-57, Nakanoshima, Kita-ku, Osaka 530, Japan.

cause mitochondrial dysfunction in many cases [6,10]. However, the precise mechanism of mitochondrial dysfunction by peroxidation during reperfusion is still unknown. With respect to peroxidative damage of mitochondria, NADPH-dependent peroxidation has been shown to decrease the electron-transfer activities of submitochondrial particles [11]. These findings strongly suggest that, in addition to increase in proton permeability, peroxidation during reperfusion causes serious damage of the mitochondrial respiratory chain, and that this type of injury is unavoidable on reperfusion of an ischemic organ. In this work, we studied the damage of mitochondria during reperfusion in relation with lipid peroxidation at the mitochondrial membrane. We found that reperfusion of ischemic liver directly suppressed mitochondrial electron-transfer activities, especially the enzymic activities of Complex I and III, as the result of lipid peroxidation.

Materials and Methods

Male Sprague-Dawley rats weighing about 200 g were used for liver perfusion studies, and those of about 350 g for isolation of mitochondria. The animals were starved for about 24 h and anesthetized by intraperitoneal injection of ketamine at 100 mg/kg body weight before use.

Perfusion of isolated rat livers. The perfusion system described by Sugano et al. [12] was used. Livers were perfused for 50 min with Krebs–Henseleit bicarbonate solution containing 5.6 mM glucose at 30°C. The flow rate was 3 ml/min per g liver, the perfusion pressure was about 10 cm H_2O, and the perfusate was saturated with 95% O_2/5% CO_2 gas mixture with a gas change device [13]. Then livers were flushed with Collins' solution [14] and incubated at 0°C for 6 h, 24 h or 48 h. They were then reperfused. The oxygen concentration in the perfusion fluid leaving the liver was measured with a Clark-type electrode and the activity of aspartate aminotransferase (EC 2.6.1.1) in the perfusate was measured to check that perfusion was appropriate.

Preparation of mitochondria and submitochondrial particles. Mitochondria were isolated from rat livers as described previously [15]. Submitochondrial particles were prepared by sonication of mitochondria by the method of Beyer [16], and were washed three times to remove superoxide dismutase and glutathione peroxidase.

Incubation of mitochondria and submitochondrial particels. Mitochondria (10 mg protein/ml) were incubated as described previously [5]. The incubation medium consisted of 0.3 M mannitol/10 mM Tris-HCl (pH 7.4)/5 mM potassium phosphate (pH 7.4)/0.1 mM EDTA/0.1% bovine serum albumin/1 mM $MgCl_2$. The mitochondrial suspension was divided in two: one half was incubated under aerobic conditions (room air), and the other under 95% N_2/5% CO_2. Under the aerobic conditions, the adequate oxygen concentration required to prevent increase in the rate of State-4 respiration [15] was maintained by shaking the mitochondrial suspension very slowly for 24 h, 48 h and 72 h at 4°C. For experiments on artificial peroxidation, submitochondrial particles, 5–10 mg protein/ml, were incubated in the incubation medium with *t*-butyl hydroperoxide at 25°C. NADPH-dependent peroxidation of submitochondrial particles were achieved in a medium containing 0.1 M mannitol/5 mM potassium phosphate (pH 7.4)/10 mM Tris-HCl (pH 7.4)/0.1 mM EDTA/1 mM ADP/0.3 mM $FeCl_3$ at 25°C. The reaction was started by the addition of NADPH (0.5 mM).

Assay methods. The rate of oxygen consumption by isolated mitochondria was measured with an oxygen electrode (PC-100, Sumitomo Electric Industries) at 25°C in reaction mixture as described previously [5]. Adenine nucleotides were determined by high-performance liquid chromatography (HPLC) as described by Watanabe et al. [4]. Malondialdehyde was measured by the method of Ohkawa et al. [17]. Cytochromes were determined from the reduced-minus-oxidized difference spectra as described [11]. Ubiquinones were extracted from mitochondria by the method of Takeda et al. [18] and measured by the method of Vadhanavikit et al. [19].

Enzymic activities were determined at 25°C: succinate oxidase and NADH oxidase were assayed polarographically with an oxygen electrode. Other enzymes were assayed spectrophotometrically: NADH-cytochrome *c* oxidoreductase from the decrease in A_{340} and increase in A_{550} [20], cytochrome *c* oxidase from thedecrease in A_{550} [21], NADH-ferricyanide oxidoreductase from the

decrease in A_{340} [22], NADH-2,6-dichloro-indophenol (DCIP) oxidoreductase from the decrease in A_{600} [22], succinate-DCIP oxidoreductase from the decrease in A_{600} [23], NADH-ubiquinone-1-oxidoreductase from the decrease in A_{340} [24], succinate-ubiquinone-2-oxidoreductase from the decrease in A_{600} [25], and dehydro-ubiquinone-cytochrome *c* oxidoreductase from the increase in A_{550} [26]. Protein was determined by the method of Lowry et al. [27] with bovine serum albumin as a standard.

Chemicals. Ubiquinone-1, and ubiquinone-2 were kindly provided by Nisshin Seifun Co.

Results

Damage of mitochondrial respiratory chain on re-oxygenation after hypothermic incubation of rat livers

There are several reports that preserved or ischemic organs show decreased rates of oxygen consumption on reoxygenation [28,29]. This was confirmed in this study: we found that the rate of hepatic oxygen consumption during reperfusion decreased to 92%, 78% and 63% of that initially after cold incubation periods of 6 h, 24 h and 48 h, respectively. Because the oxygen consumption is overwhelmingly due to mitochondrial respiration, this decrease seemed to be caused by dysfunction of mitochondria. Threfore, we examined the oxygen consumption rate of mitochondria isolated from these livers after their anoxic incubation and then reperfusion for 60 min. The rate of State-3 respiration decreased with that of total hepatic respiration and a similar decrease was also observed in uncoupler-released respiration. Unlike in the case of mitochondrial damage during warm anoxia, there was little increase in State-4 respiration in reperfused livers after prolonged cold incubation (Fig. 1). These results indicated that the mitochondrial electron-transfer system was preferentially damaged in reperfused livers after hypothermic incubation.

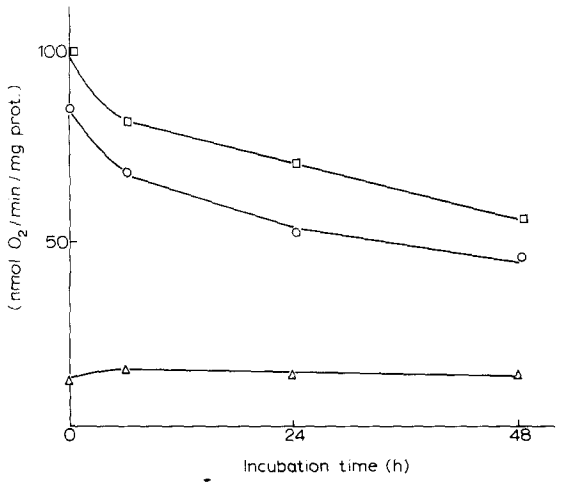

Fig. 1. Changes in mitochondrial respiration rate of reperfused livers after hypothermic incubation. Perfusion of isolated rat livers and hypothermic incubation were performed as described in Materials and Methods. Mitochondria were isolated from livers that had been reperfused for 60 min at 30 °C. □, respiration rate in the presence of uncoupler (CCCP); ○, rate of State-3 respiration; △, rate of State-4 respiration.

Damage of the mitochondrial respiration chain during hypothermic incubation of isolated mitochondria

Similar mitochondrial damage was observed when mitochondria isolated from normal rat liver were incubated under hypothermic aerobic conditions. Mitochondria incubated under aerobic conditions showed marked decrease in uncoupler-released respiration, in striking contrast to mitochondrial damage by anoxia, which showed no decrease in uncoupler-released respiration (Fig. 2A and B). Decrease of the State-3 respiration rate

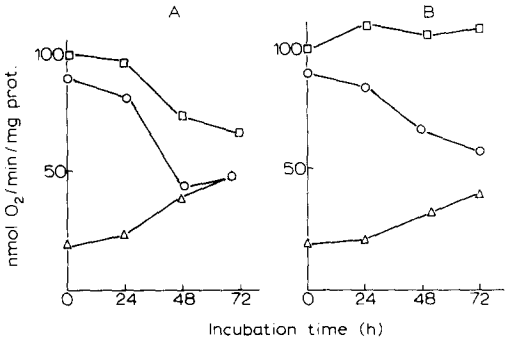

Fig. 2. Changes in mitochondrial respiration rate during aerobic and anaerobic incubations. Mitochondria were incubated as described in Materials and Methods. At the times indicated, the respiration rate of mitochondria (oxidizing succinate; A and B) was measured. (A) showed the changes during aerobic incubation. (B) showed those during anaerobic incubation. □, respiration rate in the presence of CCCP; ○, rate of State-3 respiration; △, rate of State-4 respiration.

and increase of the State-4 respiration rate were observed during both aerobic and anaerobic hypothermic incubations. The increase of the State-4 respiration rate during aerobic and anaerobic incubations was accounted for by decrease in the intramitochondrial ATP level, as described previously [4,5]. The decrease of State-3 respiration rate during anaerobic incubation has been shown to be caused by decrease of total adenine nucleotides in mitochondira [15]. In addition to decrease of total adenine nucleotides, the decrease of uncoupler-released respiration might be partly related to decrease of State-3 respiration during aerobic incubations. However, the mechanism of the repression of electron-transfer activity observed during aerobic incubation seemed to be completely different and to involve a toxic action of activated oxygen, because it did not occur under anaerobic conditions.

Changes in activities of enzyme complexes of the mitochondrial electron-transport system during hypothermic incubation

Similar decrease in respiratory activity were observed with succinate and NAD-linked substrates as respiratory substrates (data not shown). To determine the sites that were inhibited during aerobic incubation, we measured the activities of several enzymes involved in the mitochondrial electron-transport system. As shown in Fig. 3A and B, there was no remarkable change in the activity of succinate dehydrogenase (with DCIP as electron acceptor), NADH dehydrogenase (with ferricyanide or DCIP as electron acceptor), or cytochrome *c* oxidase, but NADH-cytochrome *c* oxidoreductase activity was reduced during aerobic incubation. Thus, the inhibitory site seemed to be in about the middle of the electron-transfer chain, and so the following three partial reactions involving oxidation-reduction of ubiquinone were examined. During aerobic incubation, there was no significant decrease in the activity of succinate-ubiquinone oxidoreductase, but moderate decrease in that of NADH-ubiquinone oxidoreductase, and marked decrease in that of dihydroubiquinone-cytochrome *c* oxidoreductase (Fig. 3C). In contrast to the decrease in enzymic activities, little change was observed in the contents of electron carriers, such as cytochromes and ubiquinones, during aerobic incubation (data not shown).

Increase of lipid peroxidation during aerobic incubation

Because the decreases of electron-transfer activ-

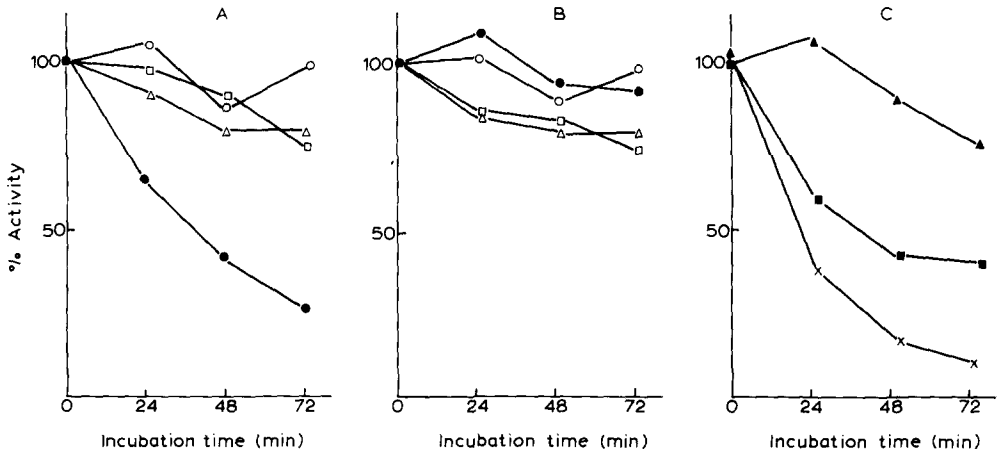

Fig. 3. Changes in enzymic activities of mitochondria in aerobic and anaerobic conditions. Mitochondria were incubated as for Fig. 2, and then solubilized by 0.1% Triton X-100 or 0.2% deoxycholate in 50 mM potassium phosphate buffer (pH 7.4) or sonicated at the times indicated. (A) and (C) show changes during aerobic incubation. (B) shows those during anaerobic incubation. Enzymic activities are expressed as percentages of the initial value (activities at zero time are given in parentheses in nmol/min per mg protein at 25°C). □, succinate dehydrogenase (93.7); ○, NADH dehydrogenase with DCIP as electron acceptor (194); ●, antimycin-sensitive activity of NADH cytochrome *c* oxidoreductase (102); △, cytochrome *c* oxidase (first order constant = 2.95/s per mg protein); ■, rotenone-sensitive activity of NADH-ubiquinone oxidoreductase (45); ▲, succinate-ubiquinone oxidoreductase (95); ×, activity of dihydroubiquinone-cytochrome *c* oxidoreductase (187).

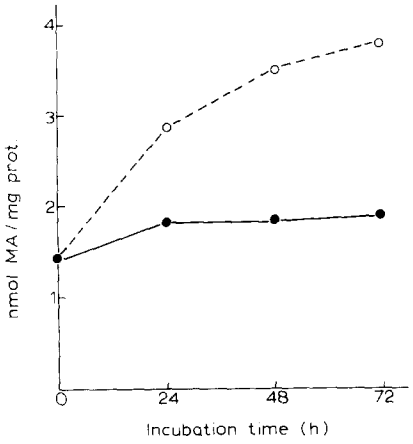

Fig. 4. Increase of lipid peroxidation during aerobic incubation. Mitochondria were incubated as described in Materials and Methods. Lipid peroxidation was expressed as formation of malondialdehyde. Incubation: ○, aerobic conditions; ●, anaerobic conditions.

ities during aerobic incubation seemed to be caused by toxic effects of activated oxygen species, we measured changes in the content of lipid peroxides. As shown in Fig. 4, malondialdehyde formation increased with time during aerobic incubation, but not during anaerobic incubation. These changes together with the absence of change in the contents of electron carriers strongly suggested that the decrease of electron-transfer activities during aerobic incubation was caused by lipid peroxidation.

Damage to electron-transfer activities of rat-liver submitochondrial particles induced by lipid peroxidation

To verify that lipid peroxidation caused the damage of the electron-transport system, we examined the toxic effects of an artificial peroxide, *t*-butyl hydroperoxide, on the respiratory activity of submitochondrial particles of rat liver. When added to the incubation mixture, *t*-butyl hydroperoxide caused dose- and time-dependent decrease in the activity of succinate oxidase (Fig. 5A). NADH oxidase was more sensitive than succinate oxidase to *t*-butyl hydroperoxide (Fig. 5B). Concomitantly with the decreases of these enzymic activities, products of lipid peroxidation, measured as malondialdehyde formation, increased (Fig. 5C). An experimental peroxide forming system, composed of NADPH, ADP, $FeCl_3$ and O_2, also strongly inhibited the activities of succinate and NADH oxidases (Fig. 6). The activity of NADH oxidase was slightly more sensitive than that of succinate oxidase to this system as it was to *t*-butyl hydroperoxide. This was probably because NADH oxidase has one more site than

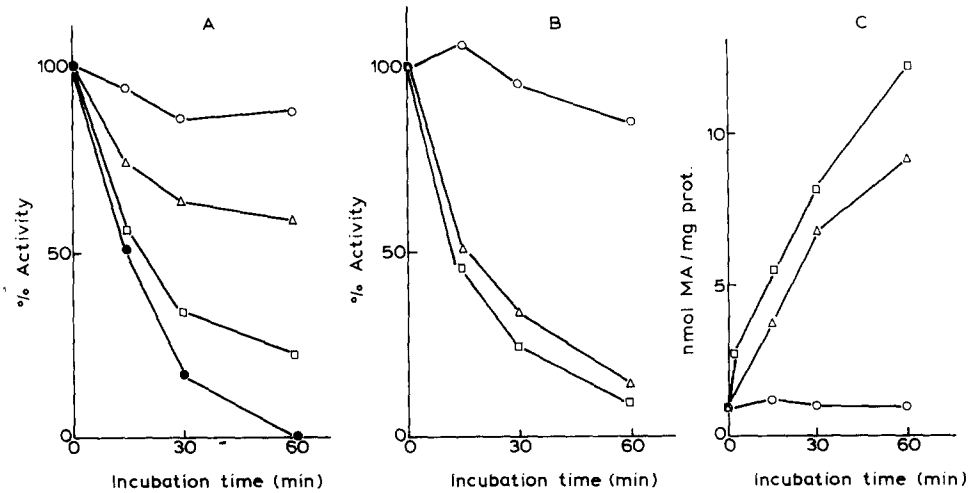

Fig. 5. *t*-Butyl hydroperoxide-induced damage of the mitochondrial respiratory chain. Submitochondrial particles were incubated as described in Materials and Methods. At the times indicated, the activities of succinate oxidase (A), NADH oxidase (B), and lipid peroxidation (C) were measured. Enzymic activities are expressed as percentages of the initial value (activities of succinate oxidase and NADH oxidase at zero time are 47.0 ± 6.50 and 124 ± 6.24 nmol O_2/min per mg protein at 25°C, respectively). ○, no addition; △, 2 mM *t*-butyl hydroperoxide; □, 5 mM *t*-butyl hydroperoxide; ●, 10 mM *t*-butyl hydroperoxide.

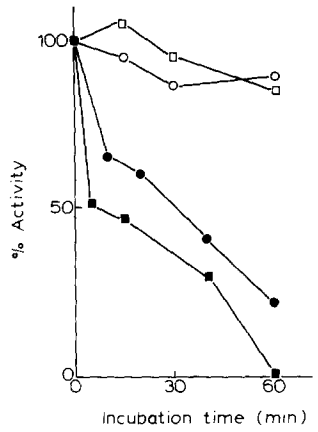

Fig. 6. Effect of NADPH-dependent lipid peroxidation on the mitochondrial respiratory chain. Submitochondrial particles were incubated as described in Materials and Methods. At the times indicated, the activities of succinate oxidase (○, ●) and NADH oxidase (□, ■) were measured. Enzymic activities are expressed as percentages of the initial value as Fig. 5. ○ and □, control; ● and ■, NADPH-dependent lipid peroxidation.

succinate oxidase that is sensitive to the peroxidation (Fig. 3A and B). These data showed that lipid peroxidation caused decrease in the electron-transfer activities of mitochondria during reperfusion and during aerobic incubation.

Discussion

The present study together with a previous study on mitochondrial damage during anoxia [1,4,5,15] showed that two different biochemical mechanisms of mitochondrial dysfunction were involved in cellular injury of ischemic liver. One mechanism acts during anoxia, and the other on reoxygenation after anoxia. During anoxia, decrease in the intramitochondrial ATP level causes release of Ca^{2+} [5], which disrupts the barrier against proton diffusion of the mitochondrial inner membrane [4]. During reoxygenation, the cells recover from anoxic damage, but they are concomitantly injured by peroxidation. Lipid peroxides generated by oxygen radicals have been found to cause various morphological and functional disturbances [7,8,30]. The present study showed that when hypothermically preserved liver was reperfused, lipid peroxidation inhibited the activity of the mitochondrial electron-transport system.

In intact cells in mammalian liver, oxygen radicals are formed at a rate of 24 nmol O_2/min per g [31], but normally these radicals are not detectable, and do not cause injury because they are quickly scavenged by a battery of intracellular defence enzymes, such as superoxide dismutase, catalase and several peroxidases [9]. In mitochondria, about 2% of the State-4 respiration rate results in production of oxygen radicals [32]. The sites where superoxide anion is generated have been shown to be Complex I [33] and Complex III [34,35]. These sites are closely related to the sites that were shown in this study to be sensitive to and injured by peroxidation. Probably in vivo oxygen radicals are degraded so rapidly that they can only react with lipids close to the site of their production. The following reasons may explain why oxygen damages ischemic cells on reoxygenation, but does not damage normal cells.

(1) Reperfusion and reoxygenation result in a burst of free radical production because of the accumulation of reduced substrates during anoxia and ischemia [9]. Actually mitochondria produce much more H_2O_2 in State-4 respiration than in State-3 [32], and electron carriers in Complex III are capable of reducing O_2 to O_2^- only when they are reduced [34]. This is probably the main reason for reperfusion injury.

(2) Substrates such as hypoxanthine, which accumulate during anoxia, are oxidized by xanthine oxidase with production of superoxide anion. In this context it is noteworthy that pretreatment with allopurinol has a protective effect against reperfusion injury [7].

(3) The supply of endogenous scavengers may decrease during ischemia. This decrease may be partly related to the observations of decrease in the cellular level of NADPH in oxidative stress [36,37], and decrease of superoxide dismutase activity during ischemia [38].

Free radicals generated during reperfusion after ischemia cause lipid peroxidation. Unsaturated fatty acids are the most susceptible to peroxidation because of thermodynamic stabilization of a free radical adjacent to an olefinic group [39]. In mitochondria, 50% of all fatty acids are unsaturated and in particular cardiolipin consists of polyunsaturated fatty acids [40]. This, in turn, suggests that mitochondrial lipids, especially

cardiolipin, are particularly susceptible to peroxidation. In fact, cardiolipin decreased rapidly in NADPH-dependent peroxidation in vitro [11]. In this connection, it is noteworthy that Fry and Green [41] have shown the absolute requirement of several mitochondrial enzymic activities for cardiolipin. The enzymic complex that requires cardiolipin involves dihydroubiquinone-cytochrome *c* oxidoreductase and NADH-ubiquinone oxidoreductase, but not NADH-ferricyanide reductase. This finding is consistent with our results. Thus we conclude that free radicals generated during reperfusion after ischemia and during aerobic incubation of mitochondria cause lipid peroxidation, probably peroxidation of cardiolipin, which leads to decreases of the enzymic activities of Complex I and III.

Acknowledgement

This work was supported in part by Grants for Scientific Research (B59480131 and B61480130) from the Ministry of Education, Science, and Culture of Japan.

References

1 Kamiike, W., Watanabe, F., Hashimoto, T., Tagawa, K., Ikeda, Y., Nakao, K. and Kawashima, Y. (1982) J. Biochem. 91, 1349–1356
2 Mittnacht, S., Jr., Sherman, C. and Farber, J.L. (1979) J. Biol. Chem. 254, 9871–9878
3 Mittnacht, S., Jr. and Farber, J.L. (1981) J. Biol. Chem. 256, 3199–3206
4 Watanabe, F., Hashimoto, T. and Tagawa, K. (1985) J. Biochem. 97, 1229–1234
5 Tagawa, K., Nishida, T., Watanabe, F. and Koseki, M. (1985) Mol. Physiol. 8, 515–524
6 Marubayashi, S., Dohi, K., Yamada, K. and Kawasaki, T. (1984) Biochim. Biophys. Acta 797, 1–9
7 Peterson, D.A., Asinger, R.W., Elsperger, K.J., Homans, D.C. and Eaton, J.W. (1985) Biochem. Biophys. Res. Commun. 127, 87–93
8 Paller, M.S., Hoidal, J.R. and Ferris, T.F. (1984) J. Clin. Invest. 74, 1156–1164
9 Fridovich, I. (1983) Annu. Rev. Pharmacol. Toxicol. 23, 239–57
10 Fuller, E.O., Goldberg, D.I., Starnes, J.W., Sacks, L.M. and Delivoria-Papadopoulos, M. (1985) J. Mol. Cell. Cardiol. 17, 71–81
11 Narabayashi, H., Takeshige, K. and Minakami, S. (1982) Biochem. J. 202, 97–105
12 Sugano, T., Suda, K., Shimada, M. and Oshino, N. (1978) J. Biochem. 83, 995–1007
13 Hamilton, R.L., Berry, M.N., Williams, M.C. and Severinghaus, E.M. (1974) J. Lipid Res. 15, 182–186
14 Collins, G.M., Petterson, T., Wichomb, W.N. and Halasz, N.A. (1984) J. Surg. Res. 36, 1–8
15 Watanabe, F., Kamiike, W., Nishimura, T., Hashimoto, T. and Tagawa, K. (1983) J. Biochem. 94, 493–499
16 Beyer, R.E. (1967) Methods Enzymol. 10, 184–194
17 Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Anal. Biochem. 95, 351–358
18 Takeda, M., Ikenoya, S., Yuzuriha, T. and Katayama, K. (1984) Methods Enzymol. 105, 147–155
19 Vadhanavikit, S., Sakamoto, N., Ashida, N., Kishi, T. and Folkers, K. (1984) Anal. Biochem. 142, 155–158
20 Hatefi, Y. and Rieske, J.S. (1967) Methods Enzymol. 10, 225–231
21 Wharton, D.C. and Tzagoloff, A. (1967) Methods Enzymol. 10, 245–250
22 Galante, Y.M. and Hatefi, Y. (1978) Methods Enzymol. 53, 15–21
23 Ackrell, B.A.C., Kearney, E.B. and Singer, T.P. (1978) Methods Enzymol. 53, 466–483
24 Hatefi, Y. (1978) Methods Enzymol. 53, 11–14
25 Hatefi, Y. and Stiggall, D.L. (1978) Methods Enzymol. 53, 21–27
26 Hatefi, Y. (1978) Methods Enzymol. 53, 35–40
27 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
28 Vladovic-Relja, T. (1984) Cryobiology 21, 511–520
29 Lannon, S.G., Tukaram, K.T., Oliver, J.A., McKinnon, K.J. and Dossetor, J.B. (1967) Surg. Gynecol. Obstet. 124, 999–1004
30 Post, J.A., Leunissen-Bijvelt, J., Ruigrok, T.J.C. and Verkleij, A.J. (1985) Biochim. Biophys. Acta 845, 119–123
31 Chance, B., Sies, H. and Boveris, A. (1979) Physiol. Rev. 59, 527–605
32 Boveris, A., Oshino, N. and Chance, B. (1972) Biochem. J. 128, 617–630
33 Turrens, J.F. and Boveris, A. (1980) Biochem. J. 191, 421–427
34 Ksenzenko, M., Konstantinov, A.A., Khomutov, G.B., Tikhonov, A.N. and Ruuge, E.K. (1983) FEBS Lett. 155, 19–24
35 Turrens, J.F., Alexandre, A. and Lehninger, A.L. (1985) Arch. Biochem. Biophys. 237, 408–414
36 Eklow, L., Moldeus, P. and Orrenius, S. (1984) Eur. J. Biochem. 138, 459–463
37 Sutherland, M.W., Nelson, J., Harrison, G. and Forman, H.J. (1985) Arch. Biochem. Biophys. 243, 325–331
38 Guarnieri, C., Flamigini, F. and Cal, C.M. (1980) J. Mol. Cell. Cardiol. 12, 797–808
39 Sevanian, A. and Hochstein, P. (1985) Annu. Rev. Nutr. 5, 365–390
40 Daum, G. (1985) Biochim. Biophys. Acta 822, 1–42
41 Fry, M. and Green, D.E. (1981) J. Biol. Chem. 256, 1874–1880