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European Journal of Pharmacology 469 (2003) 145–152
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Protective effect of melatonin against mitochondrial injury induced by ischemia and reperfusion of rat liver

Yuji Okatani[a,*], Akihiko Wakatsuki[b], Russel J. Reiter[c], Hideaki Enzan[d], Yasuyo Miyahara[a]

[a] Department of Clinical Nursing Science, Kochi Medical School, Oko, Nankoku, Kochi 783-8505, Japan
[b] Department of Obstetrics and Gynecology, Kochi Medical School, Oko, Nankoku, Kochi, Japan
[c] Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX, USA
[d] Department of First Pathology, Kochi Medical School, Oko, Nankoku, Kochi, Japan

Received 13 March 2003; accepted 21 March 2003

Abstract

Melatonin, a pineal secretory product, is a potent scavenger of a variety of free radicals. The aim of this study was to investigate the effect of melatonin on the prevention of mitochondrial injury induced by hepatic ischemia and reperfusion. Rats were subjected to 70 min of hepatic ischemia and 2 h of reperfusion. Fifteen minutes prior to ischemia and at reperfusion, animals received vehicle or melatonin (10 mg/kg body weight) intraperitoneally. In the vehicle-treated animals, the respiratory control index, ADP/O, State 3 respiration and dinitrophenol-induced uncoupled respiration decreased markedly after ischemia/reperfusion and were restored by melatonin administration. Similarly, pH change coupled with mitochondrial energy transfer was suppressed by ischemia/reperfusion with the effects being reduced by melatonin treatment. Mitochondrial lipid peroxidation was elevated in the ischemic/reperfused vehicle-treated livers, but this elevation was attenuated by melatonin. Mitochondrial glutathione peroxidase activity decreased in the vehicle-treated group with this decrease being reduced by melatonin treatment. Electron microscopic studies demonstrated that treatment with melatonin restored to near normal the ischemia/reperfusion-induced disorganization of mitochondrial structure. Melatonin protects against mitochondrial injury which reduces mitochondrial oxidative stress and improves ischemia/reperfusion-induced hepatic energy metabolism.

Keywords: Melatonin; Antioxidant; Free radical; Mitochondria; Ischemia/reperfusion

1. Introduction

Hepatic ischemia/reperfusion is a common problem encountered in a variety clinical conditions such as liver transplantation, hepatic failure after shock, and liver surgery. Ischemia/reperfusion causes functional and structural damage to liver cells (Mittnacht and Farber, 1981). Considerable evidence suggests that oxygen-derived free radicals are involved in the pathogenesis of hepatic ischemia/reperfusion injury (Kobayashi et al., 1991; Hirata et al., 1996). Pharmacological evidence including the beneficial effects of the xanthine oxidase inhibitor allopurinol (Jeon et al., 2001), or the administration of enzymes that metabolize reactive oxygen species such as superoxide dismutase (SOD) or catalase (Romani et al., 1988), support the role of oxygen species in hepatic damage. Also, other antioxidants, e.g., α-tocopherol, Trolox (a soluble analog of vitamin E) and coenzyme Q_{10} have been shown to protect the liver from ischemia/reperfusion injury (Marubayashi et al., 1985; Wu et al., 1991).

Melatonin is a ubiquitously acting direct free radical scavenger and an indirect antioxidant (Pablos et al., 1997; Reiter et al., 2000, 2001a; Allegra et al., 2003). While being highly efficient in detoxifying the devastating reactive $^{\cdot}OH$, melatonin also directly neutralizes singlet oxygen ($^1O_2$), peroxynitrite anion ($ONOO^-$) and nitric oxide ($NO^{\cdot}$) (Reiter et al., 2000, 2001b). One molecule of melatonin scavenges two $^{\cdot}OH$; the product of this interaction is cyclic 3-hydroxymelatonin (Tan et al., 1998). Recently, melatonin also was reported to directly neutralize H_2O_2 in vitro; H_2O_2 is the precursor of the $^{\cdot}OH$ (Tan et al., 2000, 2001). The product of this interaction is N^1-acetyl-N^2-formyl-5 methoxytryptamine, which is reported to be itself an efficient free radical scavenger (Tan et al., 2001). In addition to these scavenging

* Corresponding author. Tel.: +81-88-880-2561; fax: +81-88-880-2561.
E-mail address: okataniy@med.kochi-ms.ac.jp (Y. Okatani).

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doi:10.1016/S0014-2999(03)01643-1

actions, melatonin also stimulates a host of antioxidative enzymes including, glutathione peroxidase, and glutathione reductase; these actions further reduce the oxidation state of cells (Barlow-Walden et al., 1995; Antolin et al., 1996; Pablos et al., 1997; Okatani et al., 2000).

Reperfusion of the ischemic liver is associated with oxidative modifications and functional impairment of mitochondria (Kobayashi et al., 1991; Hirata et al., 1996). However, no study has yet investigated the effect of melatonin on mitochondrial respiratory function in the liver subjected to ischemia/reperfusion. Accordingly, we designed this study to clarify the effects of melatonin on post-ischemic deterioration of hepatic mitochondrial function and energy metabolism.

2. Materials and methods

2.1. Chemicals

ADP, ATP and melatonin were obtained from Sigma (St. Louis, USA). Dinitrophenol, succinate, glutamate, tetramethoxypropane, and thiobarbituric acid were purchased from Wako (Osaka, Japan). All other chemicals were of reagent grade and were locally and commercially available.

2.2. Animals

Male Sprague–Dawley rats (350 ± 20 g body weight) were housed in plexiglas cages with three animals per cage. The animal room was windowless with automatic temperature (22 ± 2 °C) and lighting controls (light on at 0700 h and off at 1900 h). A solid diet (CE-2, Clea, Tokyo, Japan) and water were provided ad libitum. The Animal Research Committee of the Kochi Medical School approved all research protocols. Melatonin was dissolved in absolute ethanol and thereafter diluted in saline (the final concentration of ethanol was less than 1%).

2.3. Experimental procedure

The animals were used after 14 days of acclimation to the animal room. The surgical procedure was performed with the rats under anesthesia [rodent cocktail given i.p. at a dose of 0.2 ml/250 g BW; the cocktail consisted of ketamine (60 mg/ml) and xylazine (8 mg/ml)]. Throughout of the experiment, body temperature was remained at 36–38 °C with the aid of a heating pad. The animals were divided into four groups: the control group; hepatic ischemia (70 min); hepatic ischemia plus 2-h reperfusion; hepatic ischemia plus 2-h reperfusion and two injections of melatonin in alcoholic saline (10 mg/kg BW). The first injection was given 15 min before ischemia and the second before reperfusion. The sham-operated control animals received the same surgical procedure as the other groups without being subjected to ischemia/reperfusion protocol; they were also given an equivalent amount of ethanolic saline solution. Following intravenous injection of sodium heparin (200 U/kg BW), all vessels (hepatic artery, portal vein, and bile duct) to the left and median hepatic lobes were occluded for 70 min using a vascular clamp. Thereafter, the clamp was removed and blood was reperfused for 2 h in each group. During the period of ischemia, 0.5 ml of saline was given i.p. to all animals every 20 min to maintain hemodynamic stability and to replace losses due to portal stasis. After 2 h, animals were killed by perfusing them with ice-cold saline via the inferior vena cava.

2.4. Preparation of mitochondria

Mitochondria were prepared from fresh livers by the method of Hogeboom (1985). The isolated mitochondria were suspended in 0.25 M sucrose, 10 mM Tris–HCl (pH 7.2), and 0.1 mM ethylenediaminetetraacetic acid (EDTA) at 20–30 mg protein/ml. Mitochondrial protein was determined using Lowry's method with bovine serum albumin as a standard (Lowry et al., 1951).

2.5. Measurement of mitochondrial respiratory activity

Oxygen consumption was measured polarographically at 25 °C using 1.0–2.5 mg protein from the fresh mitochondrial fraction in 2.0 ml of incubation medium consisting of 100 mM KCl, 0.05 mM EDTA, 10 mM Tris–HCl, and 0.1 M sucrose, at pH 7.4; this was done with the aid of a Clark-type electrode. Mitochondrial respiration was initiated by adding 150 μM ADP with 5 mM glutamate or 5 mM succinate as the respiratory substrate. Oxygen consumption measured in the presence of added ADP was defined as State 3 respiration, while that measured following the consumption of ADP was defined as State 4 respiration (Chance and Williams, 1956). The respiratory control index was calculated as a ratio of State 3 respiration to State 4 respiration, and used as a marker of mitochondrial respiratory activity. The ADP/O ratio was calculated as the ratio of the added ADP concentration to the consumption of oxygen during State 3 respiration. Uncoupled respiration was induced by adding 25 μM dinitrophenol. Mitochondrial respiration was calculated as nanomoles of O_2/min/mg protein.

2.6. Measurement of pH change coupled with mitochondrial energy transfer reaction

The pH in the mitochondrial suspension becomes alkaline when ATP formation is coupled to succinate oxidation ($ADP + Pi + nH^+ \cdot ATP + H_2O$) (Nishimura et al., 1962). The pH change in the incubation medium stoichiometry with oxidative phosphorylation was measured with a Horiba F-23 digital pH meter (Kyoto, Japan) at 25 °C in the same medium as that used for measuring oxygen uptake. The pH change coupled with ATP formation was initiated by adding 450 μM ADP and the rate of pH change per minute mg protein was determined.

2.7. Measurement of mitochondrial ATPase activity

Mitochondrial ATPase activity was determined at 25 °C by measuring the inorganic phosphate derived from ATP hydrolysis by the method of Takahashi (1995). The reaction medium (2 ml) contained 0.15 M KCl, 5 mM $MgCl_2$, and 10 mM Tris–HCl buffer (pH 7.4). Mitochondria (3 mg protein/ml) were incubated with 3 mM ATP for 10 min. The reaction was terminated by addition of 0.5 ml 40% trichloroacetic acid, and the inorganic phosphate in the reaction medium was measured spectrophotometrically at 700 nm.

2.8. Measurements of glutathione peroxidase activity and lipid peroxidation

Glutathione peroxidase activity in the mitochondria was measured using a GPx-340 kit (Bioxytech, Paris) based on the method described by Paglia and Valentine (1967). The change in absorbance at 340 nm, which results from the oxidation of NADPH, is the basis for quantitating cellular glutathione peroxidase activity. Mitochondria (10 μl) were mixed with 75 μl of assay buffer (0.05 M Tris–HCl, 5 mM EDTA) and 75 μl of NADPH reagent containing 3.2 mM glutathione, 1600 U/l glutathione reductase, and 640 μM betanicotinamide-adenine dinucleotide. Kinetic spectrophotometric analysis was initiated by adding 75 μl of 0.007% tetra-butyl hydroperoxide at 340 nm. The sample was replaced with water in the blanks. The rate of decrease in A_{340} per minute was determined by averaging the rate of change at 30-s intervals between 30 and 180 s.

Concentrations of thiobarbituric acid reactive substances were determined based on to the method of Ohkawa et al. (1979), slightly modified as previously described (Okatani et al., 2002a). In brief, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of a 0.8% TBA solution were added to the mitochondria, and the volume was brought to 4.0 ml with distilled water. The mixture was heated in an oil bath at 95 °C for 60 min. After the mixture was cooled with tap water, 5.0 ml of butyl alcohol and pyridine (15:1, v/v) were added, and the sample was shaken gently for 5 min. After centrifugation at $1500 \times g$ for 10 min, the butylalcohol-pyridine phase containing thiobarbituric acid reactive substances was separated, and absorbance was measured at 532 nm. The results were expressed as molar equivalents of malondialdehyde per milligram of protein, using malondialdehyde from tetramethoxypropane as a standard.

2.9. Electron microscopic observations

Mitochondrial pellets were pre-fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4 °C for 2 h and post-fixed in 1.5% osmium tetraoxide at 4 °C for 2 h. The samples were dehydrated and embedded in epoxy resin. Ultra-thin sections were cut using an Ultrcut-OmU_4 ultramicrotome, stained with uranyl acetate and lead citrate, and observed with an electron microscope (JEM-100S).

2.10. Statistics

All data are expressed as the mean ± S.E.M. Data were analyzed using a one-way analysis of variance (ANOVA). Scheffe's test was applied to determine differences between the groups. A level of $P < 0.05$ was accepted as indicating statistical significance.

3. Results

3.1. Oxidative phosphorylation

The results of mitochondrial respiration in the presence of glutamate or succinate and inorganic phosphate are presented in Table 1. The respiratory control index and

Table 1
Functional characteristics of rat liver mitochondria during ischemia and subsequent reperfusion

Characteristic	Control	Ischemia	Ischemia/reperfusion	Melatonin + ischemia/reperfusion
RCI	4.13 ± 0.15 (11)	1.22 ± 0.04 (11)[a]	1.55 ± 0.18 (11)	2.55 ± 0.26 (11)[b]
ADP/O	2.55 ± 0.06 (11)	1.44 ± 0.19 (11)[a]	1.71 ± 0.07 (11)	2.14 ± 0.09 (11)[b]
State 3 respiration	24.76 ± 0.99 (11)	11.14 ± 1.13 (11)[a]	12.89 ± 0.91 (11)	18.29 ± 1.98 (11)[c]
State 4 respiration	6.24 ± 0.16 (11)	8.95 ± 0.25 (11)[a]	7.83 ± 0.42 (11)	6.86 ± 0.29 (11)
DNP uncoupled O_2 uptake	21.05 ± 0.49 (11)	10.54 ± 0.63 (11)[a]	9.70 ± 0.94 (11)	14.45 ± 1.88 (11)[c]
RCI	3.69 ± 0.09 (8)	1.26 ± 0.07 (12)[a]	1.66 ± 0.14 (10)	2.41 ± 0.20 (10)[b]
ADP/O	1.78 ± 0.02 (8)	1.28 ± 0.04 (12)[a]	1.35 ± 0.04 (10)	1.55 ± 0.05 (10)[b]
State 3 respiration	45.62 ± 1.75 (8)	16.86 ± 1.40 (12)[a]	21.19 ± 2.13 (10)	31.96 ± 4.07 (10)[c]
State 4 respiration	12.40 ± 0.24 (8)	14.41 ± 0.67 (12)	14.20 ± 0.81 (10)	12.54 ± 0.60 (10)
DNP uncoupled O_2 uptake	34.35 ± 0.83 (8)	18.55 ± 2.14 (12)[a]	17.47 ± 1.61 (10)	26.22 ± 2.75 (10)[c]
pH change (× 100/min/mg protein)	3.43 ± 0.38 (8)	0.58 ± 0.04 (12)[a]	1.06 ± 0.20 (10)	2.53 ± 0.51 (10)[d]

All data are given as means ± S.E.M. Number of animals used are given in parentheses. RCI = respiratory control index. DNP = dinitrophenol.
[a] *P < 0.001 vs. control rats.*
[b] *P < 0.001 vs. rats with ischemia/reperfusion.*
[c] *P < 0.05 vs. rats with ischemia/reperfusion.*
[d] *P < 0.01 vs. rats with ischemia/reperfusion.*

ADP/O deceased significantly after ischemia. The mean values of respiratory control index and ADP/O exhibited a slight rise after reperfusion, but the rise was not statistically verified. The reductions in the respiratory control index and ADP/O during ischemia/reperfusion were significantly restored by melatonin administration. State 3 respiration also dropped markedly after ischemia and remained low during reperfusion. Melatonin significantly increased State 3 respiration compared with that in the ischemia/reperfusion group. State 4 respiration increased significantly after ischemia in the presence of glutamate and remained elevated during reperfusion. Melatonin administration did not statistically significantly alter State 4 respiration. State 4 respiration in the presence of succinate was similar to that when glutamate was used as a substitute and it was not influenced by the presence of melatonin.

3.2. Uncoupled respiration

Respiration in mitochondria is uncoupled by dinitrophenol and oxygen consumption increases, reflecting an accelerated electron transport activity. Dinitrophenol-induced uncoupled respiration showed similar patterns and values to those for State 3 respiration (Table 1). Dinitrophenol-induced uncoupled respiration decreased markedly after ischemia and remained unchanged during reperfusion. Treatment with melatonin significantly restored dinitrophenol-induced uncoupled respiration.

3.3. ATPase activity

Latent ATPase activity increased significantly during ischemia/reperfusion ($P<0.01$). Although melatonin treatment tended to reduce the latent ATPase activity ($P<0.1$, Fig. 1), the change did not reach the level of statistical significance.

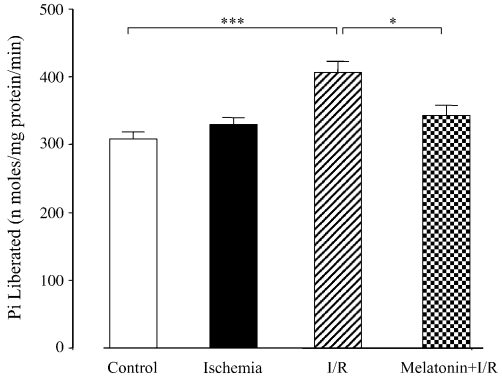

Fig. 1. Effect of melatonin (10 mg/kg BW, i.p.) on the rate ATP hydrolysis by hepatic mitochondrial ATPase after 70-min ischemia (I) followed by 2-h reperfusion (R). Values are means ± S.E.M. from *n* (at the base of each column) rats. *$P<0.1$, ***$P<0.01$.

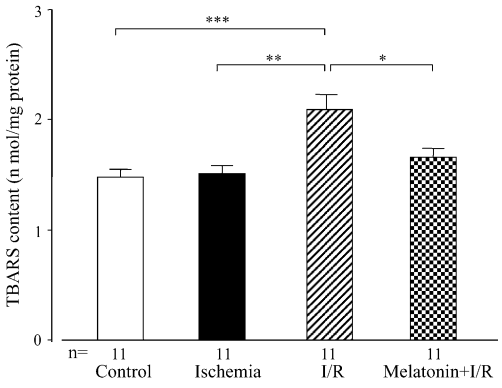

Fig. 2. Effect of melatonin (10 mg/kg BW, i.p.) on hepatic mitochondrial lipid peroxidation after 70-min ischemia (I) followed by 2-h reperfusion (R). Values are means ± S.E.M. from *n* (at the base of each column) rats. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

3.4. pH change coupled with mitochondrial energy transfer reaction

Ischemia or ischemia/reperfusion caused a drop in the pH indicating a decrease in ATP synthesis and an uncoupling of oxidative phosphorylation (Table 1). The rate of pH change was rapid after ischemia and remained unchanged during the subsequent reperfusion. Melatonin treatment significantly restored the rate of pH change. Patterns of pH change in each group were similar to those in RCI, ADP/O, and State 3 respiration.

3.5. Lipid peroxidation and GPx activity

The levels of mitochondrial thiobarbituric acid reactive substances rose as a result of ischemia/reperfusion with the increase being abolished by melatonin administration (Fig. 2). Glutathione peroxidase activity remained unchanged after ischemia, and decreased significantly during reperfusion (Fig. 3). The decrease in glutathione peroxidase activity

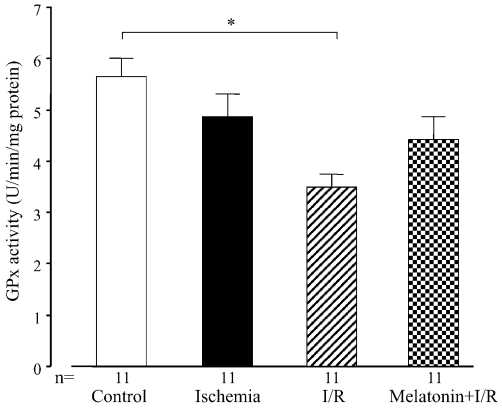

Fig. 3. Effect of melatonin (10 mg/kg BW, i.p.) on hepatic mitochondrial glutathione peroxidase (GPx) activity after 70-min ischemia (I) followed by 2-h reperfusion (R). Values are means ± S.E.M. from *n* (at the base of each column) rats. *$P<0.01$.

after ischemia/reperfusion was not influenced by melatonin treatment.

3.6. Ultrastructural observations

As shown in Fig. 4A, most of the mitochondria from the sham-operated animals were in a highly condensed form. The cristae were tightly packed and rather heavily stained. However, the mitochondria from ischemia/reperfusion-treated animals were in a swollen state with an apparent disintegration of the cristae (Fig. 4B). In contrast, the mitochondria from melatonin-treated animals were at an intermediate level; thus, some of them were in a swollen state and others appeared normal (Fig. 4C).

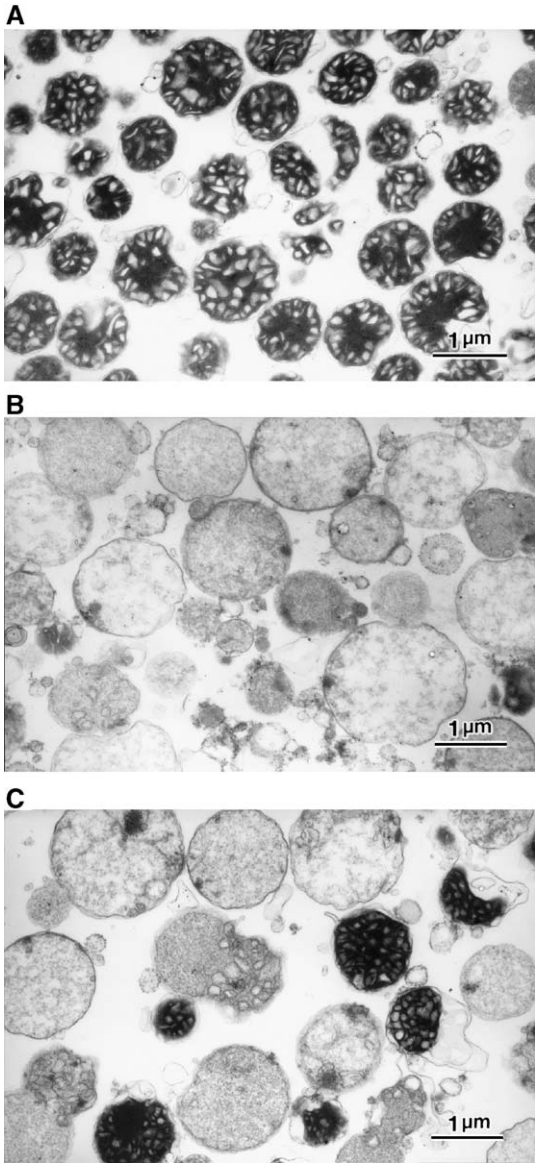

Fig. 4. Structural changes in rat hepatic mitochondria induced by ischemia/reperfusion. (A) Vehicle-treated control rat; (B) ischemia/reperfusion without melatonin treatment; (C) melatonin treatment prior to ischemia/reperfusion; magnification, × 10,000.

4. Discussion

Reactive oxygen species such as the superoxide anion radical $O_2^{\cdot -}$, H_2O_2, and hypochlorous acid are believed to be involved in the tissue destructive effects of reperfusion after ischemia (De Groot, 1994). During ischemia/reperfusion, the majority of the oxygen free radicals are produced in mitochondria or in activated granulocytes, monocytes, and macrophages (Jaeschke, 1991). This leads to molecular destruction including lipid peroxidation which causes alterations in biomembrane-associated functions and structure which causes disruption of the physiology of cells and of subcellular organelles (McCord, 1985). In the present study, mitochondrial lipid peroxidation products remained unchanged in vehicle-treated ischemic rats, but significantly increased during reperfusion.

We previously found that glutathione (GSH) levels, a major intracellular endogenous antioxidant, are reduced in the liver after a period of ischemia followed by reperfusion (Sewerynek et al., 1996). The drop in GSH levels during ischemia/reperfusion is likely due to its consumption as a result of the elevated oxidative stress. The present study shows that the activity of glutathione peroxidase, which detoxifies H_2O_2 while oxidizing GSH to GSSG, was depressed during ischemia/reperfusion. The reduction in the activity of this antioxidative enzyme probably further contributed to the resulting oxidative damage by making H_2O_2 available for conversion to highly reactive $^{\cdot}OH$.

The present study demonstrates that the respiratory control index in hepatic mitochondria from ischemia/reperfusion underwent a marked reduction due to a reduced State 3 respiration and an increased State 4 respiration. The decrease in ADP/O in mitochondria from ischemia/reperfusion was likely due to uncoupling as a result of membrane damage. Ischemia/reperfusion caused a marked drop in the pH indicating a decrease in ATP synthesis and an uncoupling of oxidative phosphorylation. The increase in State 4 respiration in mitochondria during ischemia/reperfusion may also be explained by their uncoupling. The stimulation of uncoupling induced by ischemia/reperfusion was supported by the finding of the increase in latent ATPase activity. Similar to State 3 respiration, the increased rate of oxygen consumption induced by dinitrophenol in the ischemia/reperfusion animals showed a marked reduction. This also suggests that the coupling mechanism for energy transfer reactions of the electron transport system may be altered during ischemia/reperfusion. Higher levels of oxygen free radicals are also found when the respiratory chain is inhibited (Livrea et al., 1997).

A large body of evidence suggests that a channel formed in mitochondrial membranes, identified as the permeability transition pore, is involved in cell damage associated with ischemia/reperfusion (Crompton, 1999; Fiskum et al., 1999). This channel increases the permeability of the inner mitochondrial membrane to solutes (Bernardi et al., 1994; Zoratti and Szabo, 1995). Permeability transition pore open-

ing is triggered by the association of calcium overload with an inducer such as oxidative stress or high phosphate concentrations; both these conditions are encountered during ischemia/reperfusion. The opening of this pore leads to the loss of the mitochondrial membrane potential and mitochondrial swelling which results in mitochondrial uncoupling and inhibition of ATP synthesis. Although in the current study we did not measure the mitochondrial membrane potential, ischemia/reperfusion cause an obvious disruption of mitochondrial structure characterized by swelling as shown in Fig. 4; this suggests that the permeability transition pore was opened with the alteration being at least partially prevented by melatonin.

The most important findings in the present study are that melatonin protects against ischemia/reperfusion-induced impairment of mitochondrial respiration, ATP synthesis, mitochondrial swelling, and lipid peroxidation. This indicates that mitochondria may be a major pharmacological target of melatonin. The protective effect of melatonin may be related to its potent free radical scavenging action (Reiter et al., 2001b; Poeggeler et al., 2002) or/and an inhibition of the opening of the permeability transition pore. Ischemia/reperfusion is closely associated with Ca^{2+} overload, an overproduction of oxygen free radicals, an increase in phosphate and a decrease in cellular ATP levels (Bernardi et al., 1994; Zoratti and Szabo, 1995). The permeability transition pore is highly sensitive to the redox state of mitochondria and oxidative stress triggers permeability transition pore opening. Melatonin is highly effective in scavenging $^{\cdot}OH$ and other reactive oxygen and nitrogen species (Reiter et al., 2000, 2001a). Melatonin inhibits free radical-mediated lipid peroxidation both in vivo and in vitro (Cuzzocrea et al., 2001; Tesoriere et al., 2001). We previously found that long-term treatment with melatonin protects against age-related oxidative damage of lipids, protein, DNA and mitochondrial respiratory function in the brain of senescence accelerated mice (Morioka et al., 1999; Okatani et al., 2002a,b,c). We also found a protective effect of melatonin against an impairment of cerebral mitochondrial function induced by ischemia/reperfusion in fetal rat (Wakatsuki et al., 2001). Melatonin is highly lipophilic, readily crossing a variety of membranes to enter a variety of subcellular compartments. Additionally, melatonin stabilizes cell membrane fluidity (Garcia et al., 1997), thereby preserving the functions of the membranes.

The ability of melatonin to preserve, at least in part, mitochondrial physiology under conditions of oxidative stress in consistent with the actions of melatonin at the level of this organelle as previously summarized (Acuña-Castroviejo et al., 2001, 2002). Thus, disruption of the mitochondrial respiratory cycle and ATP synthesis due to toxin exposure have been shown to be restored by melatonin administration (Absi et al., 2000; Martin et al., 2000, 2002). Under these conditions, melatonin likely reduced electron leakage and free radical generation, which would contribute to its ability to protect against molecular damage at the mitochondrial level as seen herein and elsewhere (Pappolla et al., 1999).

Polymorphonuclear neutrophil recruitment is also involved in the pathogenesis of hepatic ischemia/reperfusion injury since they stimulate inflammatory mediators such as tumor necrosis factor-alpha and interleukin 1 (Suzuki et al., 1994). In a previous study in which 1 h of reperfusion was followed by 40 min of ischemia, we found that the number of polymorphonuclear neutrophils was elevated over that of the control livers with the effect being greatly reduced by melatonin (Sewerynek et al., 1996). Recently, Rodriguez-Reynoso et al. (2001) reported that exogenous melatonin prevents alterations in the energy state of hepatocytes during ischemia/reperfusion in rat liver which is associated with a reduced concentration of tumor necrosis factor-alpha and inhibition of expression of nitric oxide synthase and nitric oxide production.

On the basis of the current findings, we conclude that exogenous melatonin is capable of preserving mitochondrial function and the energy state of cells during ischemia/reperfusion. Considering the large number of studies documenting the ameliorative effects of melatonin against ischemic/reperfusion injury in organs other than the liver (Sahna et al., 2002; Sener et al., 2002; Sun et al., 2002) along with the current findings, it seems likely that the indole may have pharmacological utility in conditions where the liver is subjected to ischemia/reperfusion damage.

Acknowledgements

This study was supported by Research Grant 11671625 from the Ministry of Education of Japan. We would like to thank Dr. Yoshihiro Hayashi (Department of First Pathology) for his help with electron microscopic analysis.

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