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ORIGINAL ARTICLE

Melatonin suppresses autoxidation and hydrogen peroxide-induced lipid peroxidation in monkey brain homogenate

Dun-Xian Tan, Lucien C. Manchester, Russel J. Reiter, Javier Cabrera, Susanne Burkhardt, Tracey Phillip, Eloisa Gitto, Malgorzata Karbownik & Qi-Dong Li

Department of Cellular and Structural Biology, The University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284-7762, USA.

Correspondence to: Russel J. Reiter, Ph.D.
Department of Cellular and Structural Biology, Mail Code 7762,
The University of Texas Health Science Center,
7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA.
TEL: 210/567/3859 FAX: 210/567/6948
E-MAIL: reiter@uthscsa.edu

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Abstract

OBJECTIVES: Melatonin, the major secretory product of the pineal gland, is known as an effective antioxidant and neuroprotector. Its neuroprotective actions and mechanisms have been documented in a variety of rodent brain models. However, little is known of melatonin's antioxidative capacity in the brain of primates. Herein, we investigated whether melatonin would suppress autoxidation and exogenous hydrogen peroxide-induced lipid peroxidation in monkey cerebral cortical homogenates. MATERIALS & METHODS: The monkey brain was dissected during routine autopsy and immediately frozen at –80°C until the experiment. A sample of cerebral cortex (50 mg) was homogenized in 1 ml ice cold phosphate buffer (20 mM, pH 7.4) at 0–4°C. Four different treatments of cerebral cortical homogenates were performed: 1) homogenates incubated in a water bath at different temperatures (4°C, 25°C or 37°C, respectively) for two hours to induce autoxidation; 2) homogenates co-incubated with different concentrations of melatonin at 37°C for 2 hours; 3) homogenates co-incubated with 1 mM vitamin C and different concentrations of hydrogen peroxide at 37°C for 1 hour to induce membrane lipid peroxidation; 4) homogenates incubated with different concentrations of melatonin and 1 mM H_2O_2 plus 1 mM vitamin C. After incubation, homogenates were analyzed for products of lipid peroxidation (malondialdehyde and 4-hydroxy-alkenals). RESULTS: The levels of lipid peroxidation products significantly increased in monkey cerebral cortical homogenates as a consequence of autoxidation or after the addition of H_2O_2 plus vitamin C. Melatonin not only suppressed the increase in lipid peroxidation induced by H_2O_2 plus vitamin C but also inhibited lipid breakdown resulting from autoxidation. The concentrations of melatonin required to suppress lipid peroxidation resulting from autoxidation or induced by exogenous oxidants in monkey cerebral cortical homogenates were in the same dose range. CONCLUSION: The results show for the first time that melatonin functions as an antioxidant and neuroprotector in primate brain tissue as was observed previously in rodent brain. The data provide information supporting the use of melatonin in the treatment of neurodegenerative

Introduction

Melatonin was identified four decades ago in extracts of the bovine pineal gland [1]. Subsequently, melatonin was found to be the major secretory product of the pineal gland in vertebrates [2]. Since melatonin is generated in a gland, regulates the seasonal reproductive activity and serves as the chemical messenger of darkness [3], it has been described as a neurohormone. A number of melatonin receptors have been identified and cloned [4, 5]. Recently, it was found that besides its receptor-mediated functions, melatonin also exhibits non-receptor-mediated actions, i.e., as a free radical scavenger [6, 7]. Since the first report of melatonin as a free radical scavenger in 1993 [6], hundreds of scientific publications have directly or indirectly confirmed this observation.

Melatonin has been found to be a potent neuroprotector in a variety of pathological brain models which involve oxidative damage. For example, melatonin protects against ischemia/reperfusion brain injury [8–10], neurodegenerative damage induced by kainic acid or quinolinic acid [11–13] and the consequences of head trauma [14] in mice and rats. It also inhibits lipid peroxidation induced by lipopolysaccharide and hydrogen peroxide in rat brain homogenates [15, 16].

Currently there is no information regarding melatonin's protective effect against lipid peroxidation in the primate brain. Considering the differences in the antioxidative status of the brain of rodents and monkeys [17] and the fact that monkey brain is presumably more similar to the human brain with regard to its antioxidative defense mechanisms, we tested whether melatonin would suppress autoxidation and hydrogen peroxide-induced oxidation in a monkey brain homogenates.

Material and Methods

Melatonin was a gift from Helsinn Co. (Biasca, Switzerland). A lipid peroxidation kit purchased from Calbiochem (La Jolla, CA) was used to measure the products of lipid peroxidation, malondialdehyde (MDA) and 4-hydroxy-alkenals (4-HDA). Other chemicals were purchased from Sigma (St. Louis, MO).

The brain of a healthy, young adult, male, green verrit monkey *(Chlorocebus aethiops)* (6 kg) was provided by the Soho Primate Research Facilities, Basseterre, St. Kitts. The brain was dissected during a routine autopsy and immediately frozen at –80°C until the experiment. Fifty mg of cerebral cortex was homogenized in 1 ml ice cold phosphate buffer (20 mM, pH 7.4) at 0–4 °C. Four different treatments of the cortical homogenates were carried out:

1) Initially, homogenates were incubated in a water bath at different temperatures (4°C, 25.0°C and 37°C, respectively) for two hours to induce autoxidation;
2) homogenates were co-incubated with different concentrations of melatonin at 37°C for 2 hours;
3) homogenates were co-incubated with 1 mM vitamin C and different concentrations of hydrogen peroxide at 37°C for 1 hour to induce membrane lipid peroxidation; 4) different concentrations of melatonin were added to the cerebral cortical homogenates that were incubated with 1 mM H_2O_2 and 1 mM vitamin C. The level of lipid peroxidation (LPO), i.e., MDA + 4-HDA, was estimated as described in a previous publication [18]. The protein concentrations were determined by the Bradford method [19]. Each treatment included 3–4 independent determinations run in duplicate. The data were expressed as means ± SE. ANOVA followed by Student's t-test was used for the statistical analysis. $p < 0.05$ was considered as statistically significant.

Results

The results show that cerebral cortical homogenates of the green verrit monkey undergo autoxidation as indicated by the increased levels of LPO products. The autoxidative response was temperature-dependent. The levels of MDA + 4-HDA in homogenates incubated at 37°C were several-fold higher than those incubated at 4°C (Fig. 1). Hydro-

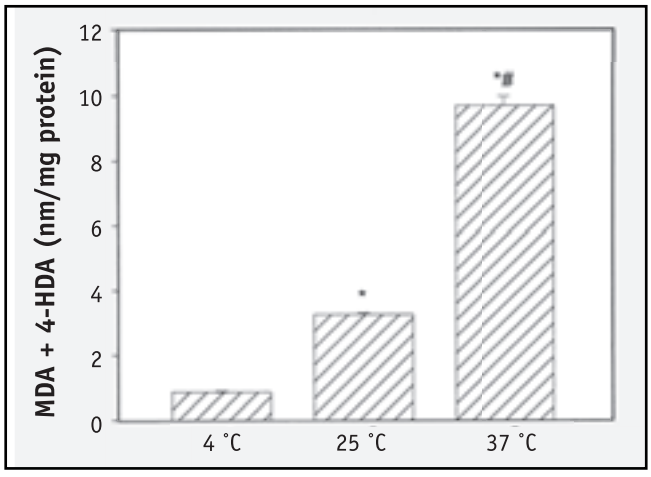

Fig. 1. Effects of temperature on lipid peroxidation (MDA + 4-HDA) resulting from autoxidation in monkey cerebral cortical homogenates. The data were collected from 4 independent studies run in duplicate at each temperature. Mean ± SE, * $p <$ 0.05 vs. 4°C group, # $p <$ 0.05 vs. 25.0°C group.

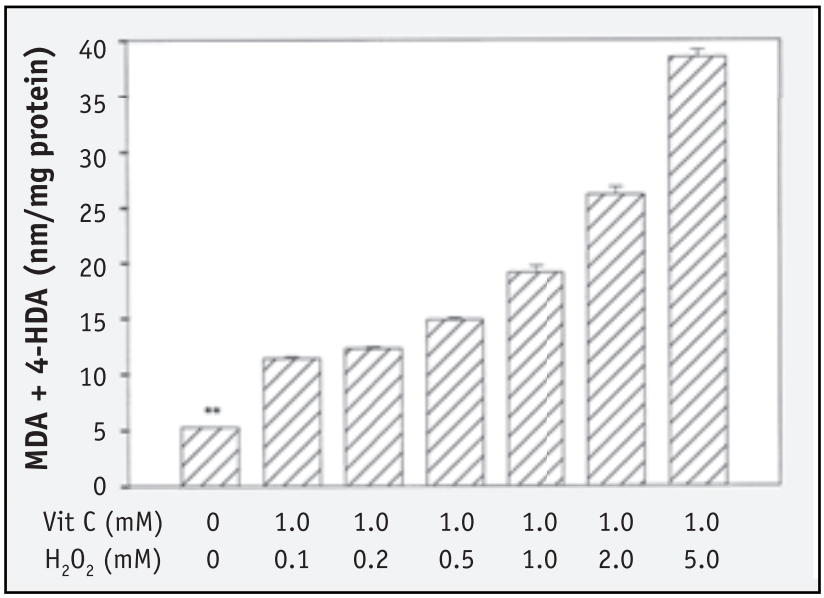

Fig. 2. Effect of H_2O_2 and vitamin C on lipid peroxidation (MDA + 4-HDA) in monkey cerebral cortical homogenates. The data were collected from 3 independent studies run in duplicate. Mean ± SE, * $p < 0.05$ vs. H_2O_2 and vitamin C treated groups.

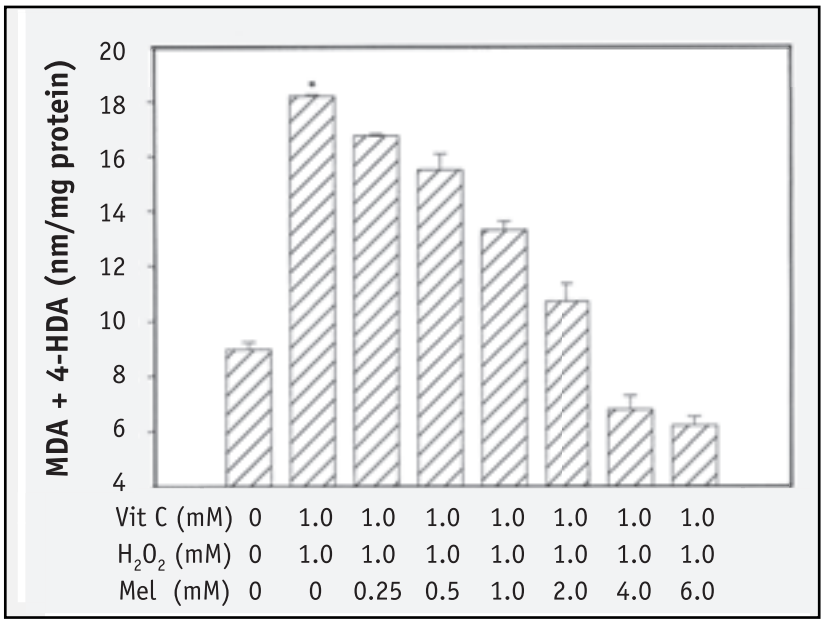

Fig. 3. The suppressive effect of melatonin on lipid peroxidation (MDA + 4-HDA) induced by H_2O_2 and vitamin C in monkey cerebral cortical homogenates. The data were collected from 3 independent studies run in duplicate. Mean ± SE, * $p < 0.05$ vs. the other groups; Mel = melatonin.

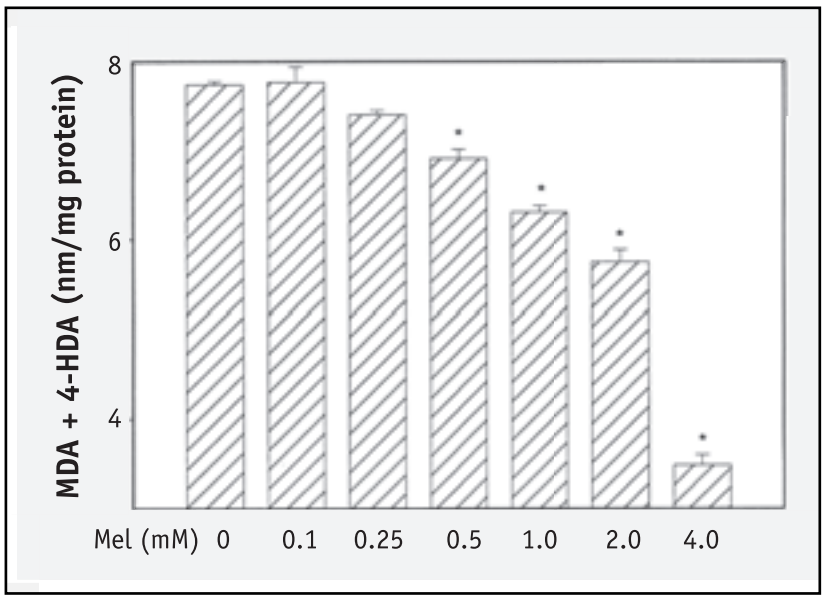

Fig. 4. The suppressive effect of melatonin on lipid peroxidation (MDA + 4-HDA) resulting from the autoxidation of monkey cerebral cortical homogenates. The data were collected from 3 independent studies run in duplicate. Mean ± SE, * $p < 0.05$ vs. Control group; Mel = melatonin.

gen peroxide plus vitamin C markedly induced lipid damage in the cerebral cortical homogenates. This damage exhibited a dose-response relationship as H_2O_2 concentrations were increased (Fig. 2). Melatonin not only suppressed LPO induced by exogenously added H_2O_2 and vitamin C (Fig. 3) but also inhibited the accumulation of MDA + 4-HDA generated by autoxidation (Fig. 4) in monkey brain homogenates. Interestingly, the concentrations of melatonin required to suppress LPO induced by autoxidation or by exogenous addition of H_2O_2 and vitamin C were in a similar dose range.

Discussion

There is extensive evidence and widespread agreement that reactive oxygen species are involved in neurodegenerative conditions such as Alzheimer's disease and Parkinson's disease. A variety of *in vivo* and *in vitro* brain models have been used to study neural damage induced by a variety of different oxidative insults. It is presumed that the monkey brain more closely mimics the human brain when compared with evolutionarily lower- ranked species in terms of its response to oxidative insults. Indeed, one report claims that the monkey brain may resist oxidative stress differently than the rodent brain [17]. For this reason, the non-human primate brain cerebral cortex was selected for the current study.

The results indicate that monkey brain cerebral cortical homogenates exhibited a strong tendency towards temperature-dependent autoxidation. This autoxidative response was readily apparent when the levels of MDA + 4-HDA were compared in homogenates incubated for 2 hours at different temperatures. Autoxidation may be related to the oxygen concentration in the medium and to the presence of endogenous oxidants [20]. It is well-known that brain tissue contains an abundance of unsaturated lipids, free iron and a diminished catalase activity [21], all of which could be associated with increased autoxidative responses. These properties of brain tissue and the aerobic incubation procedures used here made the homogenates vulnerable to autoxidation. Because of the high level of autoxidation in monkey brain cerebral cortical homogenates, they showed resistance towards further large increases in LPO when co-incubated with H_2O_2 and/or ferrous iron (data not shown). To achieve further significant increases in LPO in the homogenates, both H_2O_2 and vitamin C were required in the incubation medium. Vitamin C, under certain conditions and concentrations, is a strong pro-oxidative agent [20, 22]; it reduces ferric iron to ferrous iron. The ferrous iron donates an electron to H_2O_2 to form, in the Fenton reaction, the highly reactive and toxic hydroxyl radical (·OH). The ·OH readily damages lipids to initiate lipid peroxidation. In this study, when H_2O_2 and vitamin C were added, levels of LPO products in the homogenates were significantly increased. This increased concentration of MDA + 4-HDA corresponded well with the higher levels of H_2O_2 (Fig. 2).

To examine whether melatonin has the capacity to protect primate brain against oxidative damage, monkey cerebral cortical homogenates were incubated with vitamin C and H_2O_2 (both at 1 mM). These were the concentrations most likely to enhance LPO by a factor of two (Fig. 2). Using this model, different concentrations of melatonin were added to the homogenates. The results clearly show that melatonin inhibited LPO induced by vitamin C and H_2O_2 in cerebral cortical homogenates in a dose-response manner. In this system, LPO is believed to be initiated by the ·OH. The suppression of the levels of MDA + 4-HDA by melatonin may be directly related to its potent ·OH scavenging capacity [6]. Interestingly, melatonin inhibited autoxidation of monkey brain tissue as well as the autoxidative processes which were likely related to intrinsic oxidants present in the tissue. The concentrations of melatonin required to suppress LPO which occurred as a result of autoxidation or due to addition of vitamin C and H_2O_2 are in the same range.

This is the first report to show that melatonin prevents lipid peroxidation induced by oxidative stress in a primate brain. In this study, the melatonin concentrations used were obviously in the pharmacological range. However, the aerobically-incubated monkey brain homogenates also were not under physiological conditions and the concentrations of oxidants and pro-oxidants used in this study were far above the physiological levels. It would be unreasonable to believe that physiological levels of melatonin could detoxify pharmacological levels of toxins as existed under the conditions used herein. It has been reported, however, that physiological levels of melatonin can provide protection against brain ischemia/reperfusion injury [10] and DNA damage [23] in rodents. In one case report [24] and in one small scale clinical trial [25], melatonin at a relatively moderate pharmacological dose ameliorated the behavioral changes and halted the cognitive impairment in elderly Alzheimer patients with dementia. The improvement provided by melatonin in the Alzheimer patients was partially attributed to its antioxidant capacity and its ability to reduce neural oxidative damage induced by β-amyloid [26, 27]. Melatonin is also known to protect the brain from a number of

neural toxins [8, 11–13, 28, 29]. The present results show that melatonin inhibits both autoxidation and exogenous oxidant-induced lipid peroxidation in the primate brain and may help to explain the apparent beneficial effects of melatonin in Alzheimer patients and in other neurodegenerative conditions.

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