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Melatonin Protects Hippocampal Neurons In Vivo Against Kainic Acid-Induced Damage in Mice

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In this investigation, 40 mg/kg of the excitatory neurotoxin kainic acid (KA) was subcutaneously administered to CD2-F1 mice. In this mouse strain morphological damage induced by KA in the hippocampus was markedly concentrated in the CA3 pyramidal neurons. Neuronal injury was accompanied by several pathological neurobehavioral activities including arching of tail, tremors and seizures, and by certain biochemical changes, i.e., increased lipid peroxidation products (LPO) in the brain. When melatonin was injected intraperitoneally at a single dose of 5 mg/kg 10 min before KA administration, it significantly reduced these pathological neurobehavioral changes and almost completely attenuated the increase in LPO and morphological damage induced by KA. The neuroprotective effect of melatonin against KA-induced brain damage in mice is believed to be in part related to its oxygen radical scavenging properties as well as its antiepileptic and GABA receptor regulatory actions. Considering melatonin's relative lack of toxicity and ability to enter the brain, these results along with previous evidence suggest that melatonin, which is a natural substance, may be useful in combating free radical-induced neuronal injury in acute situations such as stroke and brain trauma as well as neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease that have free radicals as causative factors. J. Neurosci. Res. 54:382–389, 1998.

Key words: melatonin; kainic acid; hippocampus; free radical; lipid peroxidation; mice

INTRODUCTION

Kainic acid (KA) is an excitatory, neurotoxic substance. It binds and stimulates a subtype of ionotropic receptor of the brain neurotransmitter glutamate and results in transmembrane ion imbalance, especially causing calcium influx, which in turn generates reactive oxygen species including H_2O_2, superoxide anion ($O_2^{\cdot-}$) and the hydroxyl radical ($OH^{\cdot}$). These free radicals actively attack macromolecules within neurons, resulting in membrane lipid peroxidation, structural and functional changes in proteins, and DNA strand breaks, which may result in neuronal dysfunction and death. The prominent role that oxygen free radicals play in KA-induced neuronal injury is more than suggestive and indeed there is good evidence that antioxidants/free radical scavengers can counteract the damage induced by KA to neurons (Dykens et al., 1987). Schulz et al. (1995) provided direct in vivo evidence that the KA-induced neuronal damage was a result of free radicals.

It was recently reported that the pineal secretory product melatonin is a potent, endogenous $OH^{\cdot}$ scavenger (Tan et al., 1993a; Reiter et al., 1994), and a broad spectrum antioxidant (Reiter et al., 1998a); in some studies it was suggested that melatonin is more effective than either glutathione, mannitol, or vitamin E in its scavenging ability in vitro (Tan et al., 1993a; Pieri et al., 1994). Melatonin has been shown to protect against oxidative damage, such as that which occurs following carcinogen (safrole) treatment (Tan et al., 1993b, 1994), KA-induced DNA-adduct formation (Tang et al., 1998), paraquat-induced lipid peroxidation (Melchiorri et al., 1994), and free radical-induced membrane rigidity (Garcia et al., 1997, 1998). Furthermore, the neurotoxic action of KA in primary neuronal cultures is reportedly counteracted by the addition of the pineal secretory product, melatonin (Giusti et al., 1995). In a follow-up experiment, this group also found that melatonin is capable of reducing KA-induced neurotoxicity in vivo, i.e., melatonin modified the behavioral response of rats towards the neurotoxicity of KA and also prevented neuro-morphological injury in rats (Guisti et al., 1996a, b). At the same time, several other groups found that both in vivo or in

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vitro, melatonin reduced neuronal damage induced by KA as indicated by behavioral, biochemical and morphological measurements in a rat model (Melchiorri et al., 1995, 1996; Uz et al., 1996; Floreani et al., 1997).

To date, there have been no reports on melatonin's effect on neuronal injury induced by KA in a mouse model. Considering that mice are a frequently used experimental animal specie in neurological research and the fact that different species may exhibit different response towards neuronal toxicity of KA treatment, especially considering this response is a consequence of receptor-mediated free radical generation, it was deemed important to examine the effect of melatonin on KA-treated mice. Hence, in this study, we investigated melatonin's effects on neurobehavioral, biochemical, and morphological changes in mice treated with the neurotoxin KA.

MATERIALS AND METHODS

Melatonin was a gift from the Helsinn Co. (Biasca, Switzerland). Melatonin was first dissolved in absolute ethanol and then diluted with 0.9% NaCl solution. The final concentration of ethanol was 0.5% (v/v). Kainic acid was purchased from Sigma (St. Louis, MO) and was dissolved in 0.9% NaCl. Both chemicals were freshly prepared before use. Male CD2-F I mice (26–30 g) were purchased from Harlan (Houston, TX) and housed in Plexiglas cages with five animals per cage. The animal room was windowless with automatically regulated temperature (22 ± 2°C) and lighting (light on at 07.00 hr and light off at 21.00 hr daily, i.e., 14 hr light/ 10 h dark). The mice received standard laboratory chow and water ad labium. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee.

The animals were randomized into four groups. Group 1 (7 mice) received vehicle (0.9% NaCl solution contained 0.5% ethanol) alone; Group 2 (7 mice) received melatonin (5 mg/kg) ip alone; and Group 3 (15 mice) received a single subcutaneous injection of KA Group 4 (15 mice) received KA plus melatonin, i.e., mice received 5 mg/kg melatonin i.p 10 min before KA (40 mg/kg) subcutaneously administration. The volume for all of these injections was adjusted to 0.1 ml/30 g body weight.

Immediately following the injections, neurobehavioral changes were monitored. Classical signs of KA administration including arching of the tail, tremors, and seizures were selected as altered neurobehaviors; in addition the onset time of the first altered neurobehavioral sign was recorded. The observation period extended for 4 hr following the injections. The death rate at 48 hr was also recorded.

Since irreversible brain changes developed 24 hr and later after KA administration and the changes were restricted to the pyriform cortex, amygdala, hippocampus, gyrus olfactorius lateralis, bulbus olfactorius, and tuberculum olfactorium (Sperk et al., 1983), 48 hr after the injections, five mice of each group were sacrificed by decapitation. and the cerebral cortex was excised and immediately placed on dry ice and stored at -80°C for the LPO assay. On the day of the assay, 150 mg cerebral cortex from each mouse was homogenized in ice cold 50 mM Tris buffer, pH = 7.4, using a Euro Turrax T20B homogenizer. The assay used to measure level of lipid peroxidation products [malondialdehyde (MDA) and 4-hydroxyalkenals (4-HDA)] is described in a kit available from Calbiochem (LA Jolla CA). Lipid peroxidation was expressed as MDA + 4-HDA nmol per mg protein. The protein concentration was measured using the method described by Bradford (1976) using bovine albumin as the standard.

Seven days following the injections [obvious neuronal loss in the rat hippocampus is not observed until 6 days after KA injection (Giusti et al., 1996)], two mice in each group were sacrificed by decapitation. The brains were immersed in buffered neutral formalin solution containing 37% formaldehyde (100 ml), sodium phosphate dibasic (anhydrous) (6.5 g), sodium phosphate monobasic (4 g), and 900 ml of distilled water. The brains were fixed for 7 days and then transferred to a 70% ethanol solution for another 10 days. The hippocampi and surrounding tissue were dissected from the whole brain, cut, and stained with hematoxylin and eosin. The morphological assessment of neuronal damage was determined by using light microscopy.

The onset time of the first neurobehavioral change was expressed as mean ± SEM. Statistical analyses were carried out by means of ANOVA followed by the t-test. The neurobehavioral activities were expressed as average frequency of occurrence. The χ^2 test of 2 × two tables was used to obtain the statistical difference of these frequencies. The level of significance was established at $P < 0.05$.

RESULTS

Mice that received the vehicle (7 each) or melatonin injection alone (7 each) did not show any visible neurobehavioral changes and all of them survived throughout the experimental period. By contrast, all of the mice (15 each) that received KA treatment alone developed some of the neurobehavioral changes, including arching of the tail, tremors, and seizures (Table I). The death rate in mice that received KA treatment alone was 33% within 48 hr. In the KA plus melatonin-treated mice, the

TABLE I. Effects of Melatonin on KA-Induced Neurobehavioral Activities and Death in Mice*

Group	n	Incidence (%)				Onset time of first sign (min)
		Tail arch	Tremors	Seizures	Death	
Control	7	0	0	0	0	—
Melatonin	7	0	0	0	0	—
KA	15	86	100	100	33	22 ± 2.9
KA + melatonin	15	33**	40**	40**	20	46 ± 7.1*

*KA, kainic acid. The onset time is expressed as mean ± SEM.
*$P < 0.05$.
**$P < 0.001$.

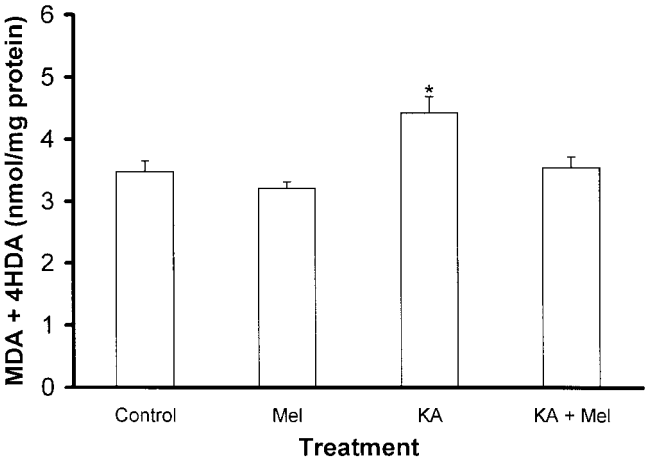

Fig. 1. Effect of melatonin on brain lipid proxidation in mice treated with kainic acid. Lipid proxidation is expressed as MDA + 4-HDA per mg protein. Data are expressed as mean ± SEM, n = 5. KA, kainic acid; Mel, melatonin; * $P < 0.05$, KA group vs. other groups.

incidence of tail arching decreased to 33% ($P = 0.009$) as compared to 86% in the KA-treated mice. Tremors and seizures decreased from 100% in the KA-treated mice to 40% ($P = 0.001$), respectively, and the death rate was reduced to 20%. In addition, the onset time of the first altered neurobehavioral sign in the KA + melatonin-treated mice was significantly delayed as compared to that of the KA-treated mice (Table I).

The level of LPO in the cerebral cortex of mice that received vehicle injection alone was 3.47±0.18 nmol/mg protein (Fig. 1). Melatonin by itself did not change the basal level of LPO (3.21 ± 0.11 nmol/mg protein). In KA-injected mice, the level of cerebral cortical LPO was increased to 4.42 ± 0.27 nmol/mg protein. This increase, when compared to the vehicle-treated mice, was greater than 27% ($P < 0.05$). In the KA plus melatonin group, the level of LPO in the cerebral cortex was reduced to 3.54 ± 0.18 ($P < 0.05$ vs. KA-treated animals). Thus, the injection of melatonin in combination with KA had significantly reduced lipid damage caused by the neurotoxin KA.

The morphological changes were consistent with these findings. Of particular interest was the status of the pyramidal neurons (CA1–CA4) in the hippocampus, since these cells are typically damaged by KA (Schwarzer et al., 1997). In the brains of mice treated with either vehicle or melatonin, the pyramidal neurons appeared intact and viable (Fig. 2A,B). By contrast, these neurons in the hippocampus of KA-treated mice appeared severely damaged. The induced neuronal damage was concentrated in the hippocampal CA3 area. The total histological architecture of the CA3 area was altered (Fig. 2C1). Virtually all of the pyrimadal neurons in the CA3 area in the KA-treated mice appeared damaged (Fig. 2C2). The cell membranes appeared to be destroyed and the nuclei of the cells were condensed and pyknotic. These changes are consistent with cell death. On the other hand, in the KA plus melatonin-treated mice, the cytoarchitecture of the hippocampal CA3 area was still intact (Fig. 2D1). Only a few damaged neurons were observed microscopically in this area (Fig. 2D2).

Fig. 2. Protective effect of melatonin on KA-induced morphological changes in the hippocampus of mice. **A1,A2:** Photographs of the hippocampus of a control mouse. A1 is an overview photograph, while A2 shows the details of pyramidal neurons in the hippocampal CA3 subregion. In these micrographs the pyramidal neurons of the hippocampus proper appear normal and the bulk of the pyramidal neurons appear functional. **B1,B2:** Photomicrographs of the hippocampus of a mouse treated with melatonin only. The neurons in these photographs appear similar to those in Figures A1 and A2, respectively. **C1,C2:** The hippocampus of a mouse treated with KA. The arrow heads (C1) identify the area where the pyramidal neurons are damaged. The morphological architecture of neurons in the hippocampal CA3 region were destroyed. Under high-power magnification, the neuronal membranes are difficult to identify, the nuclei are pyknotic and virtually all neurons appear to be in a state of degeneration. **D1,D2:** Sections of the hippocampus of a mouse treated with KA plus melatonin. While the integrity of most of the neurons seems preserved, a few neurons may exhibit some damage (D2, arrow head).

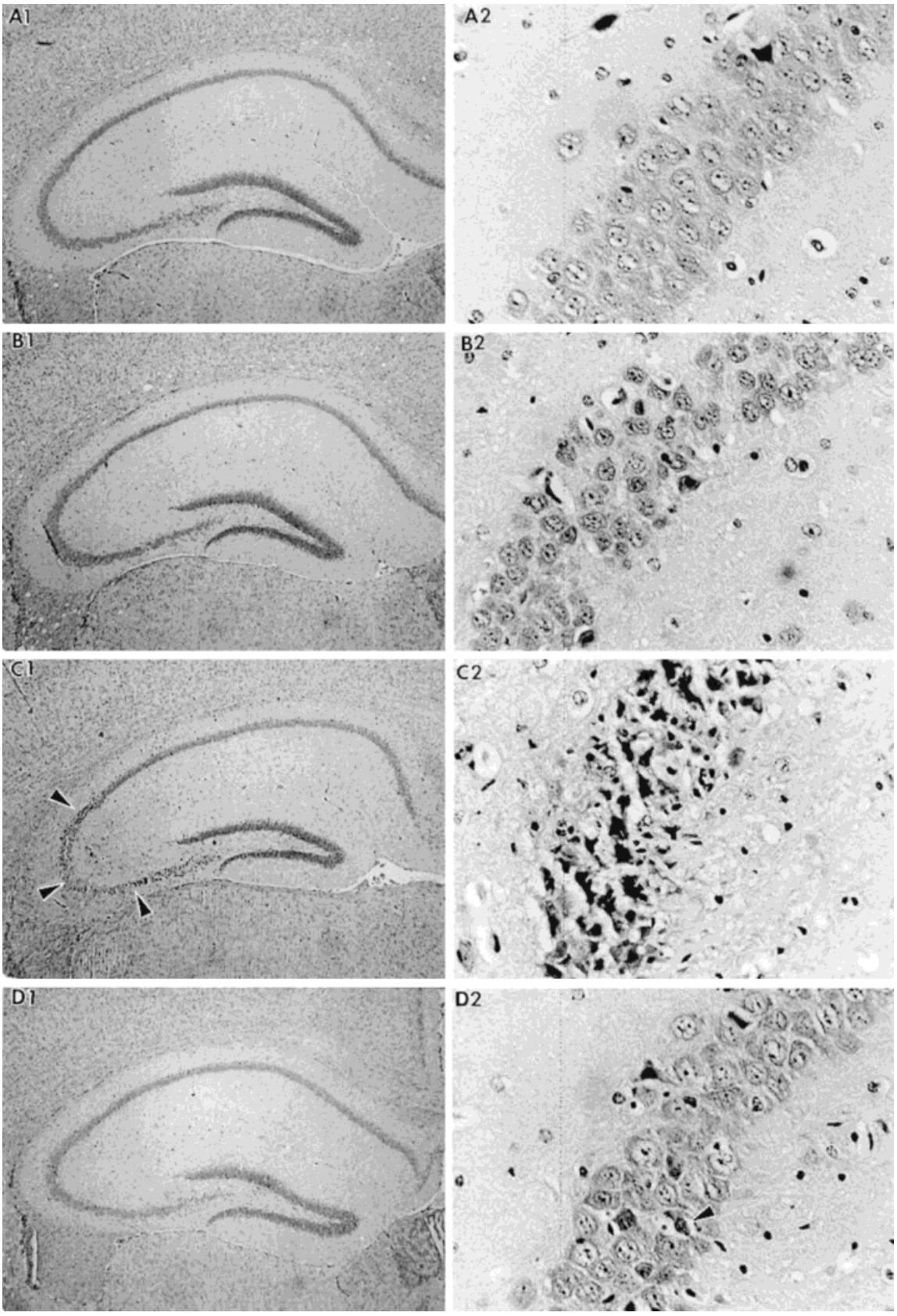

Figure 2.

DISCUSSION

In this study, we found that the pineal secretory product melatonin slightly reduced the death rate, significantly modified neurobehavioral activities, and preserved brain architectural changes induced by the subcutaneous administration of 40 mg/kg KA in mice. The protective effects of melatonin against KA-induced neuronal damage is likely due, in part, to its free radical scavenging and antioxidant properties. To test this possibility, cerebral cortical LPO was measured. LPO is the most commonly used index to indirectly indicate reactive oxygen radical damage to the cell membrane. Clearly, in the present study, LPO levels were higher in the cerebral cortex of KA-treated mice than that in the vehicle-treated mice ($P < 0.05$). In contrast, the cerebral cortical LPO levels of both the KA plus melatonin and the melatonin alone-treated mice were in the same range as those of the vehicle injected animals. This clearly indicates that melatonin prevents KA-induced lipid damage in the cerebral cortex of mice and suggests that the free radical scavenging and antioxidative properties of melatonin could provide a partial explanation for melatonin's afforded protection against KA-induced neural damage. This is consistent with previous studies in rats in which the protective effect of melatonin against toxic neuronal injury was attributed to its free radical scavenging properties (Lipartiti et al., 1996). Furthermore, based on in vitro studies, Giusti et al. (1996) have suggested that the melatonin-related neuroprotection from KA-induced excitotoxicity is not a result of an inhibitory action of melatonin on either non-N-methyl-D-aspartate ionotropic glutamate receptors, gamma-aminobutyric acid (GABA) receptors, or melatonin receptors but is attributed to its free radical scavenging function.

Perhaps melatonin's neuro-protective actions against KA may also involve several other mechanisms. Systemically administration of KA results in neurobehavioral changes in the first few hours, which includes arching of the tail, tremors, and seizures. These symptoms are accompanied with early partially reversible pathological changes in brain. These changes consist of shrinkage and pyknosis of neuronal perikarya, together with swelling of dendrites and axonal terminals as well as generalized signs of edema throughout the brain (Speark et al., 1983). Thus, KA administration represents a widely used animal model of human temporal lobe epilepsy (Schwarzer et al., 1997; Buckmaster et al., 1997). In a variety of experimental situations, melatonin is a well-documented antiepileptic substance. Decades ago, Reiter et al. (1973) reported that removal of pineal gland from parathyroidectomized rats caused seizures and often death, and suggested that melatonin may have a protective effect in the pathological neurobehavior of seizures. Champney et al. (1996) subsequently reported that the administration of melatonin diminishes seizures induced by pentylenetetrazol (PTZ) in gerbils. Recently it was reported that the systemic administration of melatonin inhibited iron-induced epileptic discharges in the rat brain and protected against iron-induced seizures (Kabuto et al., 1998) as well as seizures in a child with chronic myoclonic epilepsy (Molina-Carballo et al., 1997). Seizures are often a clinical emergency and are usually accompanied with brain damage (Lipartiti et al., 1996). Seizures, induced by either KA or PTZ, are believed to be mediated by altered GABA neurotransmission (Lipartiti et al., 1996; Champney et al., 1996). In vivo investigations have confirmed melatonin's ability to increase GABA content of the brain, influence GABA receptor activity, and exhibit anticonvulsant activity (Albertson et al., 1981; Acuna-Castroviejo et al., 1995; Champney et al., 1996).

In the current study, a interesting phenomenon was observed, i.e., in mice, the injury was localized in CA3 area of the hippocampus, where the pyrimadal cells were almost completely destroyed. By comparison, in the rat hippocampus, the neuronal morphological injury induced by KA appears to occur most markedly in the CA1 area (Sperk et al., 1983; Giusti et al., 1996), while the pyrimadal cells of CA3 area remain intact. Since neurons containing KA receptors are influenced by exogenous KA administration and neurons lacking these receptors are not involved in KA-induced injury (Cazevieille et al., 1997), it is presumed that in these two species, the KA receptor distribution in the hippocampus is different, or the receptors on different pyramidal neurons are differentially sensitive to KA. Currently, there are no reports that compare the different distributions of NMDA receptors on pyramidal neurons in the hippocampus of rats and mice.

As shown here, a single injection of melatonin (5 mg/kg), 10 min before a 40 mg/kg KA administration was sufficient to reduce KA-induced biochemical and neuromorphological changes in mice. This strongly suggests that melatonin is capable of quickly crossing the blood-brain-barrier and accumulates in significantly high concentrations in neurons to reduce any damage. Indeed, Menendez-Pelaez et al. (1993a) found that 30 min after subcutaneous injection of 0.5 mg/kg melatonin, the nuclear concentrations of melatonin in rat cerebral cortex and cerebellum were five times higher than that of control rats. This is the direct evidence showing that melatonin enters the brain with relative ease. A often raised question is how can a single dose of melatonin administration produce what appears to be such prolonged neuroprotective effects? The dosage of melatonin (5 mg/ kg) used in this study, of course, was pharmacological compared to the physiological levels of mice at the secretory peak at night (about 50 pg/ml plasma). However, melatonin's half-life in the blood is relatively short (20–40 min) while

the effects of KA can persist for weeks (Mink et al., 1991; Zaaroor et al., 1997) because it is a non-degradable glutamate receptor analogue. This indicates that the blood levels of melatonin may not be a suitable indicator of tissue levels of this constituent (Menendez-Pelaez et al., 1993b). Thus, it is possible that tissue levels of melatonin may remain higher than physiological levels when blood melatonin concentrations fall after administration of a pharmacological dose of melatonin. Another means by which melatonin could have prolonged beneficial effects is via its enhancement of other protective agents. In this context, melatonin has been reported to increase the activities of certain antioxidative enzymes, including glutathione peroxidase and glutathione reductase (Barlow-Walden et al., 1995; Pablos et al., 1998), glucose-6-phosphate dehydrogenase (Pierrefiche et al., 1995), and superoxide dismutase (Antolin et al., 1996; Rodriquez et al., 1998). Also, melatonin has been shown to inhibit a prooxidative enzyme, nitric oxide synthase (NOS) (Pozo et al., 1997; Bettahi et al., 1998). The product of NOS activity is nitric oxide ($NO\cdot$) which is involved in KA-induced neuronal damage. Besides melatonin reducing NOS, melatonin also scavenges $NO\cdot$ (Noda et al., 1998) as well as its metabolite the peroxynitrite anion ($ONOO^-$), which is also highly toxic (Cuzzocrea et al., 1998). Finally, melatonin's protective actions against toxins may also involve yet unidentified mechanisms by which melatonin may prepare cells to response to stressful stimuli.

Free radical damage in neurons has been linked to a number of age-related neurological diseases and to the normal aging processes of the central nervous system. Contrary to what might be expected, the nervous system is relatively poorly protected from and is also highly susceptible to the constant bludgeoning by active oxygen free radicals. This may relate to the fact that the brain uses a disproportionately large amount of O_2 for its size and contains high amounts of non-heme iron, ascorbic acid, and easily oxidizable lipids (Reiter, 1995). Both iron and ascorbic acid under certain conditions can serve as strong prooxidants. Alzheimer's disease (AD) is generally accepted as having a free radical component with the oxidants being generated by the neurotoxin amyloid-β (Aβ) protein. Recently, it was reported that systemic administration of KA induces Aβ in rat brain (Ong et al., 1997; Shoham and Ebstein, 1997). The mechanism by which this process occurs may be through a free radical mediated pathway which activates gene expression. The generated Aβ, in turn, accelerates further free radical generation, thus creating a reoccurring cycle. In the mouse model used in this study, the damage induced by KA was concentrated in the hippocampal CA3 subregion. It is well established that the pyramidal cells in this area are related to short-term memory functions. Damage occurring in this region of the brain results in the lost of short term memory such as in AD patients (Davies, 1985; Kesner, 1985). This is a serious problem since many factors such as stress, stroke, air pollution, and food and water contaminated with chemicals mimic the action of KA in generating free radicals. Fortunately, melatonin shows a direct function in protecting against Aβ-induced lipid peroxidation and cell damage in different cell cultures (Pappolla et al., 1997; Daniels et al., 1998). Thus, melatonin may be capable of breaking the cycle referred to previously.

Melatonin, not only protects the brain of different species against KA-induced injury but also protects against a broad spectrum of neurological injuries produced by agents or actions such as trauma (Mesenge et al., 1998), stroke (Manev et al., 1996), potassium cyanide, L-cysteine (Yamamoto and Tang, 1996a,b), and aging (Reiter, 1995). However, in most of the investigations including this study, melatonin was administered in advance of the challenge to achieve the preventive effect. Little is known as to whether melatonin still can achieve a protective effect if it was administered after such challenges. Since in the clinical situation it is likely that melatonin would be administered after the challenge, future work could be directed at administering melatonin after the damage was initiated to define the window of efficacy.

Considering melatonin's apparently low acute and chronic toxicity and its ability to readily penetrate the blood-brain-barrier and enter all neurons, melatonin may find utility in preventing and treating neurological diseases such as stroke and brain trauma as well as neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, which have free radicals as causative factors (Reiter et al., 1997, 1998b).

REFERENCES

Acuna-Castroviejo D, Escames G, Macias M, Munoz-Hoyos A, Molia-Carballo A, Aravzo M, Montes R., Vives F (1995): Cell protective role of melatonin in the brain. J Pineal Res 19:57–63.

Albertson TE, Peterson SL, Stark LG, Lakin ML, Winters WD (1981): The anticovulsant properties of melatonin on kindled seizures in rats. Neuropharmacology 20:61–66.

Antolin I, Rodriguez C, Sainz RM, Mayo JC, Uria H, Kotler ML, Rodriguez-Colunga MJ, Tolivia D, Menendez-Pelaez A (1996): Neurohormone melatonin prevents cell damage: Effect on gene expression for antioxidant enzymes. FASEB J10:882–890.

Barlow-Walden LR, Reiter RJ, Abe M, Pablos MI, Menendez-Pelaez A, Chen L.D, Poeggeler B (1995): Melatonin stimulates brain glutathione peroxidase activity. Neurochem Int 26:497–520.

Bettahi I, Guerrero JM, Reiter RJ, Osuna C. (1998): Physiological concentrations of melatonin inhibit the norepinephrine-induced activation of prostaglandin E_2 and cyclic AMP production in rat hypothalamus: A mechanism involving inhibition of nitric oxide synthase. J Pineal Res 25:34–40.

Bradford MM (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:248–254.

Buckmaster PS, Duked FE (1997): Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. J Comp Neurol 385:385–404.

Cazevieille C, Osborne NN (1997): Retinal neurons containing kainate receptors are influenced by exogenous kainate and ischaemia while neurons lacking these receptors are not: Melatonin counteracts the effect of ischaemia and kainate. Brain Res 755:91–100.

Champney T H, Hanneman WH, Legare ME, Appel K (1996): Acute and chronic effects of melatonin as an anticovulsant in male gerbils. J Pineal Res 20:79–83.

Cuzzocrea S, Zigarelli B, Gilad E, Hake P, Salzman AL, Szabo C (1997): Protective effect of melatonin in carrageenan-induced models of local inflammation. J Pineal Res 23:106–166.

Daniels WMU, van Rensburg SJ, van Zyl JM, Taljaard JJF (1998): Melatonin prevents β-amyloid-induced lipid peroxidation. J Pineal Res 24:78–82.

Davies P (1985): A critical review of the role of the cholinergic system in human memory and cognition. Ann NY Acad Sci 444:212–217.

Dykens JA, Stem A, Trenker E (1987): Mechanism of kainate toxicity to cerebellar neurons in vitro is analogous to reperfusion tissue injury. J Neurochem 491:222–1228.

Floreani M, Skaper SD, Facci L, Lipartiti M, Giusti P (1997): Melatonin maintains glutathione homeostasis in kainic acid-exposed rat brain tissues. FASEB J 11:1309–1315.

Garcia JJ, Reiter RJ, Guerrero JM, Escames G, Yu BP, Oh CS, Munoz-Hoyos A (1997): Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. FEBS Lett 408:297–300.

Garcia JJ, Reiter RJ, Ortiz GG, Oh CS, Tang L, Yu BP, Escames G (1998): Melatonin enhances tamoxifen's ability to prevent the reduction in microsomal membrane fluidity induced by lipid peroxidation. J Membr Biol 162:59–65.

Giusti P, Gusella M, Lipartiti M, Milani D, Zhu W, Vicini S, Manev H (1995): Melatonin protects primary cultures of cerebellar granule neurons from kainate but not from N-methyl-D-aspartate excitotoxicity. Exp Neurol 131:39–46.

Giusti P, Lipartiti M, Franceschini D, Schiavo N, Floreani M, Manev H (1996a): Neuroprotection by melatonin from kainate-induced excitotoxicity in rats. FASEB J 10:891–896.

Giusti P, Franceschini D, Petrone M, Manev H, Floreani M (1996b): In vitro and in vivo protection against kainate-induced excitotoxicity by melatonin. J Pineal Res 20:226–231.

Kabuto H, Yokoi I, Ogawa N (1998): Melatonin inhibits iron-induced epileptic discharges in rats by suppressing peroxidation. Epilepsia 39:237–243.

Kesner RP (1985): Correspondence between humans and animals in coding of temporal attributes: Role of hippocampus and prefrontal cortex. Ann NY Acad Sci 444:122–136.

Lipartiti M, Franceschini D, Zanoni R, Gusella M, Giusti P, Cagnoli CM, Kharlamov A, Manev H (1996): Neuroprotective effects of melatonin. In Filippini GA et al. (eds): ''Recent Advances in Tryptophan Research.'' New York: Plenum Press, pp. 315–321.

Manev H, Uz T, Kharlamov A, Joo JY (1996): Increased brain damage after stroke or excitotoxic seizures in melatonin-deficient rats. FASEB J 10 1546–1551.

Melchiorri D, Reiter RJ, Attia AM, Burgos A, Nistico G (1994): Potent protective effect of melatonin on in vivo paraquat-induced oxidative damage in rats. Life Sci 56: 83–89.

Melchiorri D, Reiter RJ, Sewerynek E, Chen LD, Nistico G (1995): Melatonin reduces kainate-induced lipid peroxidation in homogenates of different brain regions. FASEB J 9:1205–1210.

Melchiorri D, Reiter RJ, Chen LD, Sewerynek E, Nistico G (1996): Melatonin affords protection against kainate-induced in vitro lipid peroxidation in brain. Eur J Pharmacol 305:239–241.

Menendez-Pelaez A, Poeggeler B, Reiter RJ, Barlow-Walden L, Pablos MI, Tan DX (1993a): Nuclear localization of melatonin in different mammalian tissues: Immunocytochemical and radioimmunoassay evidence. J Cell Biochem 53:373–282.

Menendez-Pelaez A, Reiter RJ (1993b): Distribution of melatonin in mammalian tissues: The importance of nuclear versus cytosolic localization. J Pineal Res 15:59–67.

Mesenge C, Margaill I, Verrecchia C, Allix M, Boula RG, Plotkine M (1998): Protective effect of melatonin in a model of traumatic brain injury in mice. J Pineal Res 25:41–46.

Mink JW, Thach WT (1991): Basal ganglia motor control. III. Pallidal ablation: Normal reaction time, muscle contraction, and slow movement. J Neurophysiol 65:330–351.

Molina-Carballo A, Munoz-Hoyos A, Reiter RJ, Sanchez-Forte M, Moreno-Madrid F, Campos M, Molina-Font JA, Acuna-Castroviejo D (1997): Utility of high doses of melatonin as adjanctive anticonvulsant therapy in a child with severe myoclonic epilepsy: Two year's experience. J Pineal Res 23:97–105.

Noda Y, Mori A, Liburty R, Packer L (1998): Melatonin and its precusors exhibit nitric oxide scavenging ability. Pathophysiology 5:85.

Ong WY, He Y, Garey LJ (1997): Distribution of amyloid beta-protein immunoreactivity in the hippocampus of rats injected with kainate. J Hirnforsch 38:353–361.

Pablos MI, Reiter RJ, Ortiz GG, Guerrero JM, Agapito MT, Chuang JI, Sewerynek E (1998): Rhythms of glutathione peroxidase and glutathione reductase in brain of chick and their inhibition by light. Neurochem Int 32:69–75.

Pappolla MA, Sos M, Omar RA, Bick RJ, Dickson-Bick DLM, Reiter RJ, Ethhimiopoulos S, Robakis NK (1997): Melatonin prevents death of neuroblastoma cells exposed to the Alzheimer amyloid peptide. J Neurosci 17:1683–1690.

Pieri C, Marra M, Monori F, Recchioni R, Marcheselli F (1994): Melatonin: A peroxyl radical scavenger more effective than vitamin E. Life Sci 55:271–276.

Pierrefiche G, Laborit H (1995): Oxygen free radicals, melatonin, and aging. Exp Gerontol 30:213–227.

Pozo D, Reiter RJ, Calvo JR, Guerrero JM (1997) Inhibition of nitric oxide synthase and cyclic GMP production by physiological concentrations of melatonin in the rat cerebellum: A calmodulin-mediated mechanism. J Cell Biochem 65:430–442.

Reiter RJ (1995): Oxidative processes and antioxidative defense mechanisms in the aging brain. FASEB J 9:525–533.

Reiter RJ, Blask DE, Talbot JA, Barnett MP (1973): Nature and the time course of seizures associated with surgical removal of the pineal gland from parathyroidectomized rats. Exp Neurol 38: 386–397.

Reiter RJ, Tan DX, Poeggeler B, Menendez-Pelaez A, Chen LD, Saarela S (1994): Melatonin as free radical scavenger: Implications for aging and age-related diseases. Ann NY Acad Sci 719:1–12.

Reiter RJ, Guerrero JM, Escames G, Pappolla MA, Acuna-Castroviejo D (1997): Prophylactic actions of melatonin in oxidative neurotoxicity. Ann NY Acad Sci 825:70–78.

Reiter RJ, Tan DX, Kim SJ, Qi W (1998a): Melatonin as a pharmacological agent against oxidative damage to lipids and DNA. Proc West Pharmacol Soc, in press.

Reiter RJ, Garcia JJ, Pie J (1998b): Oxidative toxicity in models of neurodegeneration: Responses to melatonin. Restor Neurol Neurosci, in press.

Rodriquez AB, Nogales G, Ortega E, Barringa C (1998): Melatonin controls superoxide anion level: Modulation of superoxide dismutase activity in ring dove heterophils. J Pineal Res 24:9–14.

Schulz JB, Henshaw DR, Siwek D, Jenkins BG, Ferrante RJ, Cipolloni PB, Kowall N, Rosen BR, Beal MF (1995): Involvement of free radicals in excitotoxicity in vivo. J Neurochem 64:2239–2247.

Schwarzer C, Tsunashima K, Wanzenbock C, Sieghart K, Sperk G (1997): GABA(A) receptor subunits in the rat hippocampus. II. Altered distribution in kainic acid-induced temporal lobe epilepsy. Neuroscience 80:1001–1017.

Shoham S, Ebstein RP (1997): The distribution of beta-amyloid precursor protein in rat cortex after systemic kainate-induced seizure. Exp Neurol 147:361–367.

Sperk G, Lassmann H, Baram H, Kish SJ, Seitelberger F, Hornykiewicz O (1983): Kainic acid induced seizures: Neurochemical and histopathological changes. Neuroscience 10:1301–1315.

Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ (1993a): Melatonin: A potent, endogenous hydroxyl radical scavenger. Endocrine J 1:57–60.

Tan DX, Poeggeler B, Reiter RJ, Chen LD, Manchester LC, Barlow-Walden LR (1993b): The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole in vivo. Cancer Lett 70:65–70.

Tan DX, Reiter RJ, Chen LD, Poeggeler B, Manchester LC, Barlow-Walden LR (1994): Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by the carcinogen safrole. Carcinogenesis 15:215–218.

Tang L, Reiter RJ, Li ZR, Ortiz GG, Yu BP, Garcia JJ (1998): Melatonin reduces the increase in 8-hydroxy-deoxyguanosine levels in the brain and liver of kainic acid-treated rats. Mol Cell Biochem 178:299–303.

Uz T, Giusti P, Franceschini D, Kharlamov A, Manev H (1996): Protective effect of melatonin against hippocampal DNA damage induced by intraperitoneal administration of kainate to rats. Neuroscience 73:631–636.

Yamamoto HA, Tang HW (1996a): Antagonistic effect of melatonin against cyanide-induced seizures and acute lethality in mice. Toxicol Lett 87:19–24.

Yamamoto HA, Tang HW (1996b): Melatonin attenuates L-cysteine-induced seizures and lipid peroxidation in the brain of mice. J Pineal Res 21:108–113.

Zaaroor M, Starr A (1997): Experimental facial myokymia in cat. Acta Neurol Scand 95:19–22.