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Effect of melatonin and vitamin E on diabetes-induced learning and memory impairment in rats

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Abstract

Previous studies indicate that diabetes mellitus might be accompanied by a certain erosion of brain function such as cognitive impairment. The aim of this study was to examine and compare the effects of melatonin and vitamin E on cognitive functions in diabetic rats. Diabetes was induced in male albino rats via intraperitoneal streptozotocin injection. Learning and memory behaviors were investigated using a spatial version of the Morris water maze test. The levels of lipid peroxidation and glutathione were detected in hippocampus and frontal cortex. The diabetic rats developed significant impairment in learning and memory behaviors as indicated by the deficits in water maze tests as compared to control rats. Furthermore, lipid peroxidation levels increased and glutathione concentration decreased in diabetic rats. Treatment with melatonin and vitamin E significantly ameliorated learning and memory performance. Furthermore, both antioxidants reversed lipid peroxidation and glutathione levels toward their control values. These results suggest that oxidative stress may contribute to learning and memory deficits in diabetes and further suggest that antioxidant melatonin and vitamin E can improve cognitive impairment in streptozotocin-induced diabetes.

Keywords: Melatonin; Vitamin E; Diabetes; Learning; Memory

1. Introduction

Diabetes mellitus is the most common serious metabolic disorder. Diabetes is characterized by a hyperglycemia that results from an absolute or relative insulin deficiency and is associated with long-term complications affecting the eyes, kidneys, heart and nerves (Gispen and Biessels, 2000; McCall, 1992). Diabetes causes a variety of functional and structural disorders in the central and peripheral nervous systems (Biessels et al., 1994). In addition to these findings, there are electrophysiological and structural abnormalities of the brain in diabetic patients providing good reasons to believe that cognitive functions may be impaired in diabetes mellitus (Gispen and Biessels, 2000). Moderate impairment of learning and memory has been observed in adults with diabetes mellitus (Reaven et al., 1990; Ryan, 1988; Tun et al., 1990). Diabetes could conceivably lead to cognitive impairment through chronic hyperglycemia (Stewart and Liolitsa, 1999).

Streptozotocin-induced diabetes is a well-characterized experimental model for insulinopenic Type I diabetes mellitus and provides a relevant example of endogenous chronic stress (Scribner et al., 1991). It has been described that progressive structural and functional abnormalities occurred in both peripheral and central nerve fibers in experimental diabetes (Birrell et al., 2000; Sima and Sugimoto, 1999).

The role of oxidative lipid and protein damage in the pathogenesis of the diabetic state has been investigated extensively. Oxidative damage to various brain regions constitutes into the long term complications, morphological abnormalities and memory impairments (Fukui et al., 2001). The increased oxidative stress in diabetes produces oxidative damage in many regions of rat brain including the hippocampus. Enhanced formation of oxygen free radicals occurs in tissues during hyperglycemia (Baydas et al., 2002a). These oxidant radicals contribute to increased neuronal

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death through protein oxidation, DNA damage, and peroxidation of membrane lipids (Hawkins and Davies, 2001).

Previous studies from our laboratory have demonstrated that treatment of antioxidants attenuates the hippocampal neuronal cell damage diabetes-induced excitotoxicity (Baydas et al., 2002a, 2003a, 2004). Free radical scavengers have been shown to protect neurons against a variety of experimental neurodegenerative conditions.

Melatonin is a direct radical scavenger and indirect antioxidant that has previously been shown to exhibit neuroprotection under a variety of circumstances (Tan et al., 1993; Reiter, 1998; Reiter et al., 2001). This endogenously produced antioxidant is capable of scavenging both reactive oxygen and reactive nitrogen species (Tan et al., 1993; Zhang et al., 1998). Vitamin E is a lipid-soluble chain-breaking antioxidant which protects especially biological membranes from lipid peroxidation (Hong et al., 2004). The present study examined whether learning and memory deficits induced by diabetes could be prevented and/or reversed with melatonin or vitamin E treatment.

2. Materials and methods

2.1. Animals and treatment

Male Wistar rats (weighing 250 g, Firat University Research Unit, Elazig, Turkey) were housed four per cage and maintained on a 12 h–12 h light–dark cycle in an air conditioned constant-temperature (24±1 °C) room, with free access to food (normal rodent food) and water. The rats were randomly divided into two groups at the start of the experiment. Control rats ($n=10$) were injected with the vehicle alone. Diabetes was induced by a single intraperitoneal injection of 60 mg/kg body weight streptozotocin. Streptozotocin (Sigma Chemical Co, St Luis, MO, USA) was dissolved in a sodium citrate buffer (pH 4.5). Blood glucose concentrations were determined 3 days after streptozotocin injection. Rats with blood glucose levels above 250 mg/dl were declared diabetic. Diabetic rats were randomly assigned to three groups: the first group received daily melatonin at a dose of 10 mg/kg (Mel group; $n=10$). Melatonin was dissolved in a small amount of ethanol and then diluted with saline. Final concentration of ethanol was 2% and the volume of melatonin solution injected daily was 0.5 ml. The melatonin dose used in this study was chosen on the basis of our previously published experiments (Baydas et al., 2002b, 2003b). The second group received daily vitamin E at a dose of 100 mg/kg (vit E group; $n=10$) as described previously (Baydas et al., 2002a). The third group received the vehicle alone (Streptozotocin group; $n=10$). Each animal's body weight and diabetic state were reassessed after 7 weeks just prior to killing the animals. All protocols described were reviewed and approved by the Local Institutional Committee for the Ethical Use of Animals.

2.2. Morris water maze test

Animals were tested in a spatial version of Morris water maze test as described previously (Morris et al., 1982; Baydas et al., 2005a). The Morris water maze consisted of a circular water tank (120 cm diameter, 50 cm height) that was partially filled with water (25 °C). Milk powder was used to render the water opaque. The training started by acclimating the rat to the task environment with 2 days of free-swimming in the pool with no platform. Each session lasted for 2 min. The pool was divided virtually into four equal quadrants, labeled N–S–E–W. A platform (10 cm diameter) was placed in one of the four maze quadrants (the target quadrant) and submerged 1.5 cm below the water surface. The platform remained in the same quadrant during the entire experiment. The rats were required to find the platform using only distal spatial cues available in the testing room. The cues were maintained constant throughout the testing. The rats received four consecutive daily training trials in the following 5 days, with each trial having a ceiling time of 60 s and a trial interval of approximately 30 s. The rat had to swim until it climbed onto the platform submerged underneath the water. After climbing onto the platform, the animal remained there for 30 s before the commencement of the next trial. The escape platform was kept in the same position relative to the distal cues. If the rat failed to reach the escape platform within the maximally allowed time of 60 s, it was gently placed on the platform and allowed to remain there for the same amount of time. The time to reach the platform (latency in seconds) was measured.

2.3. Probe trial

A probe trial was performed wherein the extent of memory consolidation was assessed. The time spent in the target quadrant indicates the degree of memory consolidation that has taken place after learning. In the probe trial, the rat was placed into the pool as in the training trial, except that the hidden platform was removed from the pool. The time of crossing the former platform quadrant and the total time of crossing all quadrants were recorded for 1 min.

To test possible deficits in sensorimotor processes, rats were tested in the water maze with a visible platform on a new location on the final day of training (Kamal et al., 2000). For the visual test, the black target platform was placed inside the pool 1 cm above the water line. Latency times to reach the platform were recorded for each trial.

All rats were fasted overnight and then sacrificed by decapitation. The brain was removed and the hippocampus and frontal cortex were dissected for the biochemical studies. Samples were kept at −70 °C until the measurements were performed.

2.4. Protein, lipid peroxidation and glutathione assays

Protein determinations in the homogenates were performed according to the Lowry procedure using a protein assay kit (Sigma, St. Louis, MO, USA). Tissue lipid peroxidation (malondialdehyde+4-hydroxyalkenals) was determined using a LPO-586 kit (Oxis, Int. Inc. OR, USA), the method is based on a reaction of *N*-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals at 45 °C. One molecule of malondialdehyde or 4-hydroxyalkenals reacts with two molecules of *N*-methyl-2-phenylindole to yield a stable chromophore with maximal absorbance at 568 nm. Glutathione levels were determined according to the method of Ellman (1959).

Table 1
Blood glucose levels (means±S.E.M.) in the four groups of rats at the onset and at the end of the experiment

Treatment	Glucose (mg/dl)	
	Onset of study	End of study
Control	120±3.1	124±3.2
Streptozotocin	123±3.2	452±15.0[b]
Melatonin	119±3.0	437±14.1[b]
Vitamin E	121±3.1	422±14.4[b]

[b] $P<0.001$ vs. onset values.

2.5. Statistic

Data are presented as means±S.E.M. Between group differences in passive avoidance test and biochemical data were analyzed by a one-way analysis of variance (ANOVA) with the post hoc Duncan's multiple range tests. Between group differences in latencies were analyzed by the analysis of variance for repeated measurements (ANOVAR) followed by Fisher's post hoc test for all groups.

3. Results

At the onset of the study all animals had equivalent blood glucose levels (Table 1). At the conclusion of the experiment, glucose concentrations were highly significantly elevated in the blood of the streptozotocin-treated rats relative to those in the controls. These high levels were not significantly altered in animals that received melatonin or vitamin E throughout the experimental period.

It is implied that chronic hyperglycemia is accompanied by an increase in oxidative stress markers such as lipid peroxidation products, protein oxidation and DNA oxidation. In the present study we investigated the levels of lipid peroxidation in hippocampus and frontal cortex of diabetic rats. The lipid peroxidation levels in the hippocampus and cortex from diabetic rats significantly increased, whereas the glutathione levels decreased. Treatment with melatonin or vitamin E returned the levels of lipid peroxidation and glutathione toward their control values (Table 2).

Distances traveled to find the hidden platform during the acquisition phase of the experiment are present in Fig. 1. The mean escape latency for the trained rats decreased from 43 to 6 s over the course of the 20 learning trials. The mean latencies in all groups were similar in the first trial, which suggests that their motor performance (ability to swim) was unaffected by the hyperglycemia, whereas the streptozotocin group tended to use more time than controls in following trials. Diabetic animals showed a lower ability to find the platform and learn its location in the 5th day of training. This poorer performance was partly prevented by the chronic treatment with melatonin or vitamin E. Both melatonin and vitamin E treatment significantly prevented the increase in latency to find the platform in the 3rd and 5th days of training ($P<0.01$ and $P<0.05$ respectively).

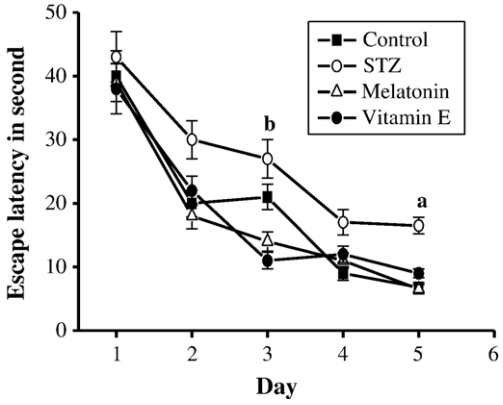

Fig. 1. Effect of diabetes and treatment with melatonin or vitamin E on the performance of spatial memory acquisition phase in rats. Data are expressed as mean±S.E.M. for 10 animals in each group. [a]$P<0.05$ and different from control, melatonin and vitamin E groups on the 5th day and [b]$P<0.01$, different from both melatonin and vitamin E groups on the 3rd day of the training sessions, (ANOVA).

Data from the probe trial of the Morris water maze study, which measures how well the animals had learned and consolidated the platform location during the five days of training, indicated significant differences between the groups (Fig. 2). Diabetic rats spent less time in the target quadrant than the control group ($P<0.01$). On the other hand, the rats treated with melatonin or vitamin E spent significantly more time in the target quadrant than the diabetic group in the probe test ($P<0.05$). The water maze parameters can be modified to test for sensorimotor impairments. All groups were submitted to a test of their ability to escape to a visible platform. The performance of all the groups of rats in the trial with the visible platform was not significantly different.

4. Discussion

Streptozotocin-induced diabetes is a well-documented model of experimental diabetes. Streptozotocin-diabetes provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia (Low et al., 1997). The roles of

Table 2
Lipid peroxidation (malondialdehyde+4-hydroxyalkenals) and glutathione levels in the hippocampus and the frontal cortex from control, streptozotocin, melatonin and vitamin E groups

		Control	Streptozotocin	Melatonin	Vitamin E
Lipid peroxidation	Cortex	3.15±0.13	4.95±0.13[b]	4.00±0.12[d]	4.13±0.14[c]
	Hippocampus	3.00±0.12	4.70±0.14[b]	3.75±0.12[d]	3.80±±0.12[d]
Glutathione	Cortex	420±13	370±12[a]	430±13[c]	485±13[d]
	Hippocampus	415±14	380±14[a]	470±15[d]	500±16[e]

[a]$P<0.05$, and [b]$P<0.001$ vs. control values; [c]$P<0.05$, [d]$P<0.01$, and [e]$P<0.001$ vs. streptozotocin group.

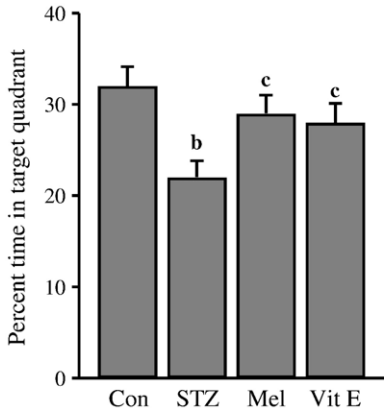

Fig. 2. Effects of diabetes and treatment with melatonin or vitamin E on the mean percentage time spent in the target quadrant in which the platform had previously been located during acquisition. Melatonin and vitamin E significantly inhibited diabetes-induced memory deficits. Data are expressed as mean ± S.E.M. for 10 animals in each group. $^{b}P<0.001$, different from control group; $^{c}P<0.05$, different from both the melatonin and vitamin E groups. Mel: melatonin; vit E: vitamin E.

oxidative stress and antioxidants in nerve damage have been studied extensively in experimental diabetes and diabetic patients (Baynes, 1991). Due to the hyperglycemia associated with diabetes, enhanced formation of reactive oxygen and RNOS occurs; this contributes to the increased neuronal death by oxidizing proteins, damaged DNA, and augmented levels of lipid peroxidation products in cellular membrane (Hawkins and Davies, 2001; Luxford et al., 2000). In the present study, streptozotocin treatment significantly increased malondialdehyde+4-hydroxyalkenals levels in the brain areas studied. These results confirm our previous reports that streptozotocin-induced diabetes is accompanied by an increased generation of reactive species (Baydas et al., 2002a; Celik et al., 2002; Baydas et al., 2005b). One reason for the elevated lipid peroxidation in streptozotocin-induced diabetes is the reduction in the levels of glutathione, a potent endogenous antioxidant. In agreement with the previous findings herein we found that untreated diabetes caused lower levels of glutathione (Baydas et al., 2002a; Tachi et al., 2001).

In the present study we indicated that both melatonin and vitamin E significantly reduced lipid peroxidation in the hippocampus and frontal cortex. Furthermore, we found that a decrease in brain glutathione was reversed by the administration of melatonin or vitamin E. These marked protective effects of melatonin and vitamin E against oxidative stress observed in this study are consistent with the previously published reports (Baydas et al., 2002a; Celik et al., 2002).

In the current study we have further examined the effects of treatment with melatonin or vitamin E on the learning and memory performance in diabetic rats. Previously, deficits in water maze learning have been demonstrated in streptozotocin-induced diabetic rats (Biessels et al., 1996). Deficits in learning in the diabetic rats were associated with the changes in hippocampal synaptic plasticity (Biessels et al., 1998; Baydas et al., 2003c). However, oxidative stress may also contribute to the learning and memory deficits during hyperglycemia. Oxidative damage to the rat synapse in the cerebral cortex and hippocampus has been previously reported to contribute to the deficit of cognitive functions (Fukui et al., 2001, 2002). Therefore, antioxidants might be of general use in the prevention of the neurodegeneration and cognitive functions associated with diabetes. The present study showed that treatment with vitamin E prevented the learning and memory deficits induced by streptozotocin-diabetes. Vitamin E may act as antioxidant to reduce oxidative damage to the synapses in hippocampus therefore improves learning and memory deficits (Fukui et al., 2002).

In the current experiment, it was also found that melatonin significantly ameliorated the cognitive impairment, reduced lipid peroxidation, and increased glutathione levels in diabetic rats. The exact mechanism of melatonin in preventing learning and memory deficits are still in debate. Shen et al. (2002) have postulated that melatonin's ability in improving the cognitive functions is related to its antioxidant action. In addition, El-Sherif et al. (2003) have suggested that melatonin may modulate specific forms of plasticity in hippocampal neurons. In the current study both oxidative stress and deficits in learning and memory were prevented by the treatment with melatonin and vitamin E suggesting that oxidative stress was probably involved in the diabetes-induced cognitive deficits.

Furthermore, as we found in the present study, the endogenous biologic antioxidant glutathione is also reduced in the streptozotocin-diabetic rats (Table 2). Reductions in the levels of this antioxidant might be reasonable factors to cause elevated oxidative stress and this, in turn, may lead to impairments in the learning and memory performance seen in diabetes. It has been speculated that decreased glutathione brain level coupled with oxidative stress may be responsible for the induction of age-related cognitive deficits (Shukitt-Hale et al., 1998; Raghavendra and Kulkarni, 2001). Melatonin and vitamin E due to their potent antioxidant nature can spare the endogenous glutathione depletion, and thereby can reverse cognitive deficits in diabetes mellitus. Herein we also compared the effects of melatonin and vitamin E on the learning and memory performance. There was no significant effect between these two antioxidants on cognitive ability, however the vitamin E dose used in the present study was ten times higher than the melatonin dose.

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