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Research report

Melatonin prevents memory impairment induced by high-fat diet: Role of oxidative stress

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ABSTRACT

Consumption of high-fat diet (HFD) induces oxidative stress in the hippocampus that leads to memory impairment. Melatonin has antioxidant and neuroprotective effects. In this study, we hypothesized that chronic administration of melatonin can prevent memory impairment induced by consumption of HFD. Melatonin was administered to rats via oral gavage (100 mg/kg/day) for 4 weeks. HFD was also instituted for the same duration. Behavioral studies were conducted to test spatial memory using the radial arm water maze. Additionally, oxidative stress biomarkers were assessed in the hippocampus. Results showed that HFD impaired both short- and long- term memory ($P < 0.05$), while melatonin treatment prevented such effects. Furthermore, melatonin prevented HFD-induced reduction in levels of GSH, and ratio of GSH/GSSG, and increase in GSSG in the hippocampus. Melatonin also prevented reduction in the catalase activity in hippocampus of animals on HFD. In conclusion, HFD induced memory impairment and melatonin prevented this impairment probably by preventing alteration of oxidative stress in the hippocampus.

1. Introduction

Consumption of high-fat diet (HFD) is common in most of world societies [31,45,85]. HFD is associated with increased risk of metabolic disorders such as diabetes, obesity and cardiovascular disease [19,28,50–52,64]. Recent researches suggest that HFD can have profound effects on the brain and can lead to cognitive impairment [5,6,11,12,22,42,65,76,82]. Additionally, HFD contributes to decline in cognitive function during aging [18,42,56], stress [4,5], sleep deprivation [10] and accelerates dementia in Alzheimer's disease [35,82].

The mechanism of HFD in decreasing cognitive function is not fully understood. Several studies have reported that HFD increases oxidative stress [10,11,49,51,52,90]. Excessive consumption of HFD increased the production of free radicals that causes lipid peroxidation and alters the structural components of blood brain barrier [10,37].

Melatonin is a natural substance found in plants, animals and fungi [21,71]. Melatonin is secreted from the pineal gland in a circadian fashion [27]. The concentration of melatonin increases nocturnally and decreases when exposed to light [69]. Melatonin is secreted into the blood, brain, tissues and cerebrospinal fluid [26]. Melatonin is an effective antioxidant because it can scavenge oxidative molecules such as superoxide anions and detoxify oxygen and nitrogen-based toxic reactants [3,30,70]. Furthermore, Melatonin enhances the glutathione

antioxidant system [3,60,63,74]. The protective effect of melatonin has been previously shown during chronic sleep deprivation-induced cognitive decline [15,89]. In this study, we evaluated the protective effect of chronic melatonin treatment on chronic HFD-induced memory impairment using the radial arm water maze (RAWM). We also carried out molecular enzymatic assays to determine some molecular targets for the studied effect of HFD and/or melatonin.

2. Methods and materials

2.1. Animals and housing conditions

Adult male Wister rats weighting 160–200 g, were used in this study. The animals were housed in plastic cages (5/cage) and were kept on a 12:12-h light–dark cycle (lights on at 7 AM), under hygienic condition and at 24° C with free access to food and water. All animal experiments were done during the daylight and were approved by Animal Care and Use Committee (ACUC) at Jordan University of Science and Technology, Irbid, Jordan.

2.2. Animal groups and diets

The rats were randomly divided into four groups (n = 12-16):
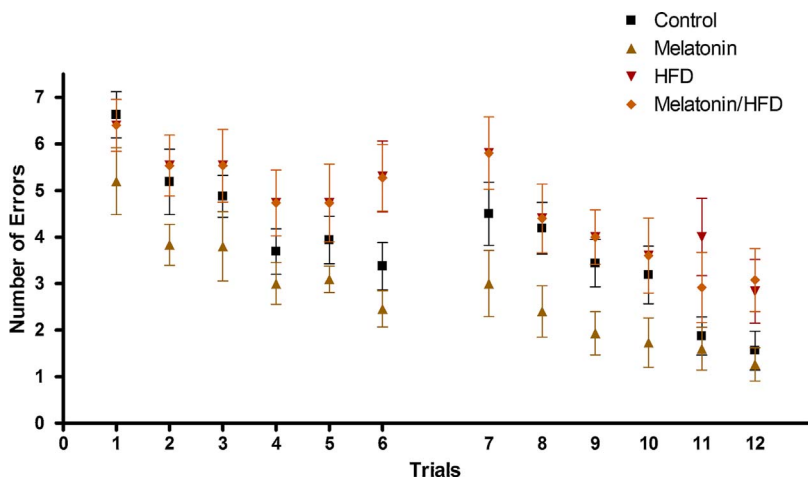
Control, Melatonin, high-fat diet (HFD) and HFD with melatonin (Melatonin/HFD). HFD and Melatonin/HFD groups were fed high-fat diet for 4 weeks. HFD contained (gm%): total fat (25%) including 11% unsaturated fats from butter and soybean oil, carbohydrates (44%) from starch, protein (18%) from casein, and 13% fibers, ash and other ingredients. The remaining groups were fed conventional diet containing (g%): 5% total fat including 2% unsaturated fats, 62% carbohydrates, 20% proteins, 13% fibers, and ash and other ingredients. In both diets, casein was the main source of proteins, butter and soybean oil were the main source of fats, and starch was the main source of carbohydrates. Both diets contained similar amount of standard vitamins and mineral mix with all essential nutrients [6,10,11,51]. Food was provided ad libitum for the duration of the experiments.

2.3. Melatonin administration

Melatonin was dissolved in distilled water and dimethylsulfoxide (DMSO) (each 100 mg of melatonin dissolved in 0.5 ml water and 0.5 ml DMSO). Melatonin (100 mg/kg) was given by oral gavage to Melatonin and Melatonin/HFD groups [15]. The Control and HFD groups received vehicle treatment. HFD and/or melatonin treatment were started on the same day and continued for 4 weeks and throughout the day of behavioral test.

2.4. Behavioral test

The RAWM was used to test spatial learning and memory among all groups of animals [1,7,8,24,38]. This model details were previously described [7,16,55,58].

2.5. Hippocampus dissection

Rats were killed after 4 week of HFD and/or melatonin treatment [4,39]. In brief, rats were killed by decapitation using guillotine and the brain was removed immediately from the skull and placed on a filter paper soaked in normal saline over a glass plate filled with crushed ice. The hippocampus was isolated, kept in Eppendorf tubes and preserved in liquid nitrogen, then at −80C until analysis.

2.6. Calorimetric assays

The hippocampus tissues were homogenized as described previously [7]. Total protein concentrations were estimated using commercially available kit BioRAD procedure (Hercules, CA, USA). The oxidized glutathione (GSH) and reduced glutathione (GSSG) were assayed according to manufacturer's instructions (Glutathione Assay Kit, Sigma-Aldrich Crop, MI, USA). Activity of superoxide dismutase (SOD) was measured in the hippocampus using SOD assay kit (Sigma-Aldrich Crop, MI, USA). Activity of catalase was determined in the hippocampal homogenate using Catalase Assay Kit (Cayman Chem. Com. Ann Arbor, MI, USA). Thiobarbituric acid reactive substance (TBARS) levels in hippocampus were measured using TBARS Assay Kit (Cayman Chem. Com. Ann Arbor, MI, USA). All procedures were performed according to kits' instructions and the absorbance was measured using an automated reader (Bio-tek Instruments, Highland Park, Winooski, USA).

3. Statistical analysis

All statistics were carried out using the GraphPad Prism (4.0) computer program (LA Jolle, CA). Comparisons of the number of errors for RAWM experiments (learning phase) were made using two-way AVOVA; followed by the Bonferroni posttest. Time (repeated measures factor) and treatment (between-subjects factor) groups were the independent variables. Comparisons of memory tests and immunoassays results were made using one-way AVOVA; followed by the Bonferroni posttest. $P < 0.05$ was considered significant. All values are represented as mean ± standard error of the mean (SEM).

4. Result

4.1. Melatonin protects against HFD-induced impairment of short- and long-term memory

At the beginning of training, the rats made high number of errors and with time the errors decreased ($P < 0.05$ within each experimental group) with no significant difference among experimental groups in each of the learning trials (treatment: $F_{(3,647)} = 2.75$, $P > 0.05$; time: $F_{(11,647)} = 12.41$, $P < 0.05$; interaction: $F_{(33,647)} = 0.34$, $P > 0.05$; Fig. 1).

At both short- and long- term memory tests done 30 min and 5 h after the end of the learning trials, the errors committed by the HFD group were significantly higher than the errors in the Control, Melatonin, and Melatonin/HFD groups (short-term memory test: $F_{(3,81)} = 117.8$, $P < 0.05$; long-term memory test: $F_{(3,78)} = 42.5$, $P < 0.05$; Fig. 2). No significant difference was found in the number of errors among Control, Melatonin, and Melatonin/HFD groups ($P > 0.05$; Fig. 2).

4.2. Melatonin prevents HFD-induced elevation hippocampus oxidative stress

The HFD resulted in a significant reduction in GSH levels as compared to Control, Melatonin and Melatonin/HFD groups ($F_{(3,50)} = 10.6$, $P < 0.05$; Fig. 3A). It also resulted in a significant elevation in GSSG