Melatonin Improves Learning and Memory Impairment in Chronic Stress Rats

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Abstract. This study aimed to observe the effect of melatonin on chronic stress rats. 60 male rats were randomly divided into five groups: control group (CON group), chronic stress group (CS group), melatonin high dose group (HMT group, 10mg/kg), melatonin middle dose group (MMT group, 5mg/kg), and melatonin low dose group (LMT group, 1mg/kg). Melatonin was daily intragastrically administrated for 4w with stress induction. Ability of spatial learning and memory of rats were determined by morris water maze. Concentration of Ca²⁺, contents of nitric oxide synthase (NOS) and nitric oxide (NO), and the number of CA3 neurons in hippocampus were tested. Compared with CON group rats, ability of learning and memory were significantly decreased, concentration of Ca²⁺ and contents of NOS and NO in the hippocampus were significantly increased in CS group rats. The number of CA3 neurons in hippocampus was decreased in CS group rats. As compared with CS group rats, ability of learning and memory were improved in MMT group and LMT group rats, and concentration of Ca²⁺, contents of NOS and NO in the hippocampus were significantly decreased, and the number of CA3 neurons was decreased in hippocampus. It shows that melatonin improving learning and memory impairment is likely by reducing the calcium and NO in hippocampus.

Introduction

There is an intense competition society, and stress is widespread in everyday, which produce a threat to the health of body and mind [1]. Studies [2] showed when people or animals were in a strong acute stress or long-term chronic stress state, their ability of learning and memory was affected. Reports [3] showed that the hippocampus is mostly associated of the learning and memory. Previous studies showed that chronic stress lead to atrophy and reduced excitability of the CA3 neurons in hippocampus [4], which could be the causes of the disorder spatial learning and memory.

Melatonin originated from the pineal gland [5] that easily passes the blood-brain barrier [6]. Melatonin has an antioxidant that may reduce lipid peroxidation and oxidation-based neurotoxicity [7]. It was also demonstrated that melatonin can influence cognitive functions. It was suggested that melatonin can modulate plasticity of hippocampal pyramidal neurons [8, 9], and may remodel structure of synapse during memory and learning. However, if the melatonin can improve learning and memory deficiency of chronic stress rats is not clear. So we studied the possible mechanisms of melatonin in learning and memory deficiency of chronic stress rats.
Material and Methods

Animals
60 Wistar male rats aged 10-12w were obtained from the Laboratory Animal Center of Jilin University, China (Certification No. SCXK(Ji)2007-0003). Animals were housed in standard temperature (22±1°C), humidity (40-50%) and light-controlled conditions (12 h light/dark cycle). All animals were housed in the facility under these conditions for 1w prior to experiments.

Stress Model
Rats were randomly divided into CON, CS, HMT, MMT, and LMT group. CS group, HMT group, MMT group and LMT group received one or two of the following uncertain stress randomly everyday for 4 w [10]: 30 min shaking with frequency 160 Hz; 1 min tail clamp; 5 min swimming at 4°C; 24 h food deprivation; 18 h water deprivation; and night or reversed day lighting conditions; 17 h of 45°cage tilt. HMT group, MMT group and LMT group rats also underwent intragastrically administration of melatonin (10 mg/kg, 5 mg/kg, 1 mg/kg) daily for 4w with stress induction. CON group and CS group rats received intragastrically administration of saline (equal volume) daily for 4w with stress.

Apparatus and reagents
Morris water maze was purchased from Taimeng (China). Fluorescence spectrophotometer was purchased from Hitachi (Japan). Melatonin was obtained from Sigma Aldrich (American). Fura-2/AM was purchased from Beijing Bo Leide Biotechnology (China). NOS kit was obtained from Huazhong University of science and technology (China).

Morris Water Maze Assay
Morris water maze is a diameter of 120 cm and height of 50 cm circular water tank. It is equally divided into four quadrants (A, B, C, D). A diameter of 10 cm platform is fixed in one of quadrant. The water surface is higher than the platform 1.5 cm. Rats searching the platform from 1th day to 6th day. Record latencies (the time of rats reaching the platform) in each trial. The animals need to find the platform within 60 sec. The platform was removed on the 7th day. Record the ratio of crossing the target platform quadrant and the total time for 60 sec.

Ca²⁺ Assay
Brains were quickly removed, then bilateral hippocampus were respectively separated, and one side of it was to make cellsuspension. Samples were loaded by Fura-2/AM for 30 min at 37°C, centrifuged 11000xg for 10 min, determined by dual wavelength. The emission wavelength was 509 nm, and excitation wavelength was 340 nm and 380 nm. Fluorescence spectrophotometer was applied to measure resting fluorescence.

NO and NOS Assay
After morris water maze test, brains were quickly cut, and bilateral hippocampus were respectively separated, and one side of it was to make hippocampal homogenates. NO were tested by a fluorometric determination. The activity of NOS was tested by non-isotope method (According to the kit method).

Test the Number of CA3 Neurons
After morris water maze test, brains were removed, and bilateral hippocampus were respectively separated, and other side of it was were fixed by formaldehyde. Nissl staining. The number of neurons were counted in x400 fields.

Statistical Analysis
Analyzed the data by SPSS16.0. Presented as mean ± standard deviation (SD). Determined by one-way analysis of variance and displayed by GraphPad Prism 6 software.
Results

Results of Morris Water Maze

Latency (the time for finding the platform) of CON group rats was longer than CS group rats ($P<0.01$). After melatonin treatment, latency was shorter than CS group rats ($P<0.01$) (Figure 1A). Percentage of time in quadrant was shorter in CS group rats ($P<0.01$). Melatonin treatment rats were significantly increased percentage of time in quadrant ($P<0.01$) (Figure 1B).

Ca$^{2+}$ Concentration in Hippocampal Neurons

Compared with the CON group rats, concentration of Ca$^{2+}$ in hippocampus was higher than CS groups rats ($P<0.01$). Concentration of Ca$^{2+}$ in hippocampus of MMT and HMT group rats, was significantly reduced than CS group rats ($P<0.01$) (Figure 2).

Content of NO and activity of NOS in Hippocampal Neurons

Content of NO and activity of NOS in hippocampus were significantly higher in the CS groups rats than CON group rats ($P<0.01$), were significantly reduced in the MMT and HMT group rats than CS group ($P<0.01$). (Figure 3).

The Number of Hippocampal CA3 Neurons

The number of CA3 neurons in hippocampus was significantly decreased in the CS groups rats than CON group rats ($P<0.01$), was significantly increased in the MMT and HMT group rats than CS group rats ($P<0.01$). (Figure 4).

Discussion

Morris water maze is an important research method for spatial learning and memory, which can accurately reflect the ability of spatial learning and memory. Our studies found that chronic stress rats had a spatial cognitive impairment by morris water maze test, while the intragastric administration of melatonin significantly relieved cognitive impairment. Which is consistent with others studies [11]. The results show that spatial cognitive performance of chronic stress rats that underwent daily intragastric administration of melatonin (10 mg/kg, 5 mg/kg) for 4w were improved.
There is an important role of free radicals play in the process of stress. Previous studies showed that chronic stress lead to atrophy and reduced excitability of the CA3 neurons in hippocampus [4], which could be the causes of the disorder spatial learning and memory. The reason of this atrophy may be contribute to reduce Gamma amino butyric acid (GABA), increase glutamate (Glu) release, and N-Methyl D-Aspartate receptors [12-14]. So, stress may activate Glu-Ca\(^{2+}\)-NOS-NO route in hippocampus, and the excessive NO make toxic effect on hippocampal neurons. This study showed that the Ca\(^{2+}\), NOS and NO were increased in the CS group, and the number of CA3 neurons were decreased in hippocampus. These results are in agreement with previous studies.

Melatonin has been suggested as an antioxidant that may reduce lipid peroxidation and oxidation-based neurotoxicity [7]. It was also demonstrated that melatonin can influence cognitive functions. It was suggested that melatonin can modulate plasticity of hippocampal pyramidal neurons [8, 9], and can remodel structure of synapse during memory and learning. In this study, our research shows that melatonin can improve learning and memory impairment in chronic stress rats, and can reduce the NO, NOS and Ca\(^{2+}\), and can increase the number of CA3 neurons in hippocampus. It shows that the mechanism of melatonin to improve learning and memory is extremely likely by reducing the Ca\(^{2+}\) and NO in hippocampus. However, the precise mechanism need to be further investigated. Our result demonstrates that melatonin can improves learning and memory impairment in chronic stress rats. The mechanism of which may be through improve Glu-Ca\(^{2+}\)-NOS-NO route in hippocampus.

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References


