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Original Article

The effects of exogenous melatonin on morphological changes in locus ceruleus nucleus Characterized by REM sleep deprivation

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Abstract

Background: Neurodegeneration in the locus coeruleus (LC) has been documented in several central nervous system (CNS) neurodegenerative diseases and sleep deprivation. In this study, we investigated the possible role of melatonin in reversing cognitive dysfunction induced by SD in rats.
Methods: The aim of this work was to determine if REM sleep deprivation would induce morphological changes in the brains of rats. The effects of REM sleep deprivation on the nuclear volume of neurons from the locus coeruleus, the main noradrenergic nucleus in the brain.
Results: The results obtained showed that REM sleep deprivation significantly decreased the number of neurons in the locus coeruleus.
Conclusion: A change in cell nuclear volume suggests a change in its metabolic activity, therefore, our data provide an anatomical basis for further studies of neuron's morphology in brain structures after REM sleep deprivation.

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Introduction

Melatonin (N-acetyl-5-methoxytryptamine) or "dark hormone", is a neurohormone secreted by the pineal gland and also by other organs as instance the retina, gut, skin, platelets and bone marrow (1-6). Melatonin secretion is related to the duration of dimness hence the secretion occurs at night and synthesis of melatonin inhibits in the presence of light(1). Physiological function of melatonin consists of: Interference in the transmission of circadian rhythms information(1), acts as an antioxidant, anti-inflammatory(2), neurodegenerative and neuroprotector agent(3), reduce the cell apoptosis in the CNS[1](4). According to the papers, application of exogenous melatonin has significant reduction effects on neural death (5, 6) because it has been recognized as an "internal sleep facilitator" and hence the exogenous melatonin is useful in the treatment of insomnia and adjustment of circadian cycle and assuagement of disorders(7). Sleep is a state of muscle relaxation and reduced perception of environmental stimuli. It has a critical action for brain function and performance. Mammalian sleep has been divided into REMS[2] and non-REMS(8). REM sleep is an exclusive phase of sleep characterized by random movement of the eyes, reduction of muscle tone, inclination to dream and propagation of low-voltage brain waves(9). Non-REM sleep charactrized by no eye movement, no dreaming occurrence, no paralyzing the muscles and reorganization of person mind(10, 11). REM sleep is a protective factor to defends neurons from damage and apoptosis(12) and RSD[3] has detrimental effects on neural health, neural cytomorphology and structural protein leading to some neurodegenerative disorders and

[1] Central nervous system

[2] Rapid eye movement sleep
[3] REM sleep deprivation

neuronal apoptosis(2, 12-14). The apoptosis due to RSD occurs in Locus Coeruleus nuclei. The LC[4], a complex of principally norepinephrine neurons, is located in the pons and the anterior end of the fourth ventricle(15). In the present study, we attempt to discover the role of exogenous melatonin in neuron apoptosis of LC nuclei.

Patients and Methods

Animals and grouping

The experiments were carried out on 40 three-months old male Wistar rats obtained from the Pasteur Institute of Iran, weighing 250-300g. Animals were housed individually in a room under controlled with 12-hour light/dark cycle and temperature (23 ± 2ºC). Food and water were provided ad libitum until the animals were sacrificed. Rats were divided randomly into five different groups of eight rats each: 1) control group with no REM-SD and no melatonin injection 2) the first group of test receives 144 hours RSD 3) the second group of test receives pre-treatment melatonin a week before 144 hours RSD administration 4) the third group of test receives post-treatment melatonin a week after 144 hours RSD administration. All groups were treated for 13 consecutive days being sacrificed by decapitation

Melatonin administration

Melatonin was dissolved in absolute ethanol and was given a dose of 20mg/kg/day intraperitoneal injection once at the end of the biologic night for seven days. The volume of melatonin solution injected was 1 ml.

REM sleep depriviation

Rats in the RSD groups were applied sleep deprivation by well-established platform approach. The cube contains a rod with a platform on top of that, surrounded by water 1 cm below the platform top. In this situation, the rats are unable to completely relax the large muscle groups without falling from the platform, getting wet, and waking. Control rats were placed platforms which exposed them to the same experimental environment as rats placed on small platforms but without the REM sleep deprivation or melatonin. The water in the was changed daily. The rats were placed on the platform for 144 hours and thus the REM sleep deprivation applied.

Animal surgery

After administration of RSD and 24 hours of the last dose in post-treatment melatonin group the rats were killed by inhalation of anesthesia gas and were perfused by the 10% formalin thus the whole body fixation applied. The brains were removed and fixed in the same fixative for 48 hours to be prepared for histological procedures.

Histological procedures

After removal, the brains were embedded in paraffin according to routine histological procedures. The anatomical extensions of LC were defined based on the rat brain atlas of Paxinos and Watson(16). Cutting started from the 9/48 to 10/32 Bregma point in 6 µm consecutive frontal sections, which were grouped in sets of 4 slices per slide by the rotary microtome (Lenca IRM 2235 Germany) to cover the LC nuclei area. The slices were stained with Nissl to detect neurons. Nissl bodies are a large granular body found in neurons can be demonstrated by a method of selective staining as Nissl staining, using an aniline stain to label extra nuclear RNA granules. This staining method is useful to localize the cell body, as it can be seen in the soma and dendrites of neurons, though not in the axon or axon hillock. Due to RNA's basophilic properties, it is stained blue by this method. The effects of melatonin on RSD were analyzed using histological procedures, Nissl staining and Image J software.

Statistical analysis

Statistical significance was evaluated using a one-way analysis of variance (ANOVA). All values are expressed as the mean±SD. The differences were considered significant at $p<0.05$.

Results

Melatonin increased cell number and volume of LC after RSD

The stereology study revealed that after 144 hours of RSD treatment the cell count is reduced in contrast with a control group(Fig. 1 & 2A). In the group treated with melatonin, the cell nuclei appear increased in number and present a basophilic related to rough endoplasmic reticulum, free ribosomes and protein synthesis. RSD group shows the loss of both count of cells and volume of the nucleus. The morphology of the damaged cell observed in the group treated with melatonin.

[4] locus coeruleus

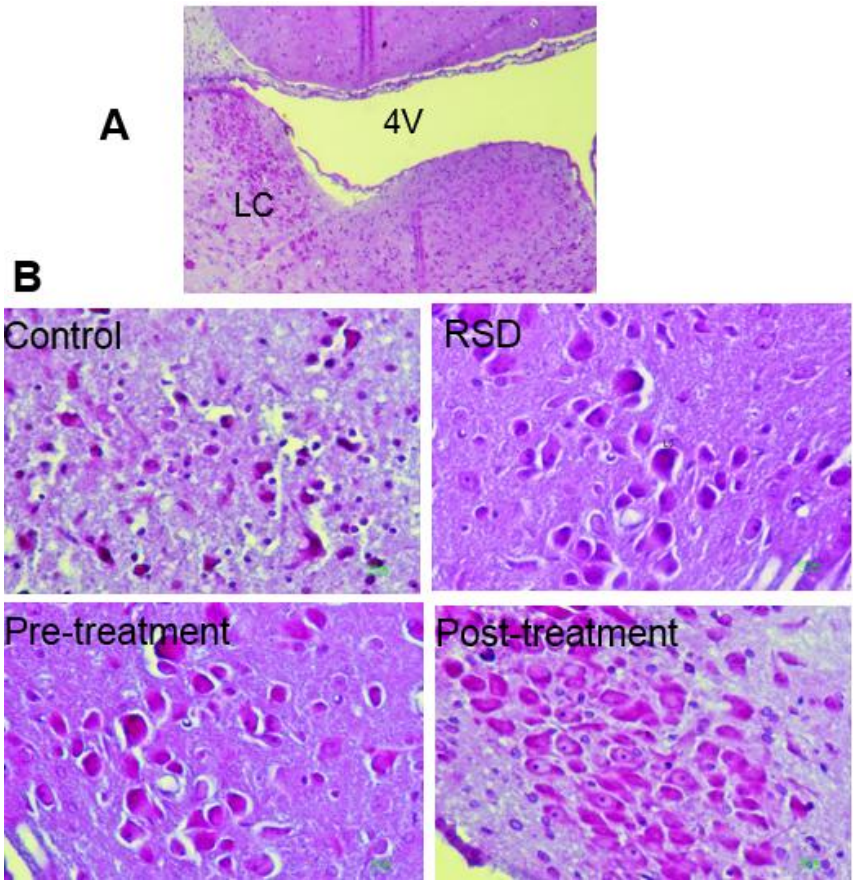

Figure.1. Nissl stainig, (A) locus coeruleus(LC) and fourth ventricle(4V), (B) study groups. Magnification: A 10× and B 40×

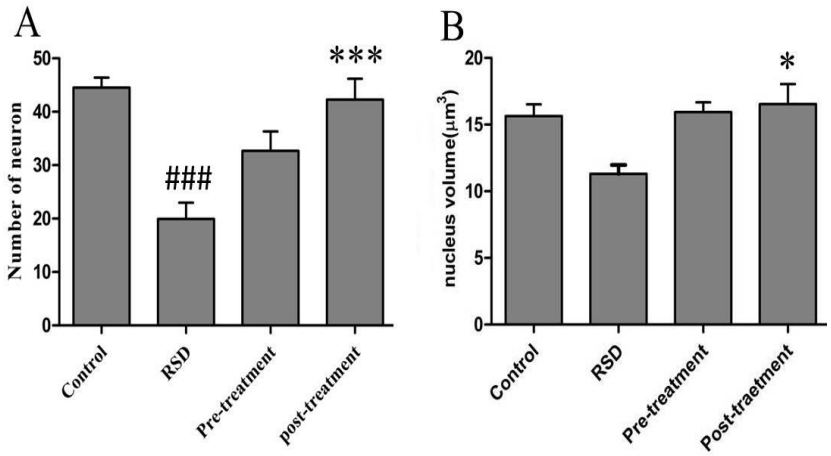

Figure.2. Stereology analysis of LC after REM sleep deprivation and treatment by melatonin. (A) Number of neuron in LC befor and after treatment of melatonin, (B) nucleus volume. * $P<0.05$ significant different between treated groups and RSD group # $P<0.05$ significant different between control group and RSD group.

Discussion

In the present study, we demonstrated that the neuroprotective effect of melatonin on attenuation of apoptosis in LC after REM sleep deprivation. Our results showed that the number of neurons in LC was reduced. After RSD the neurons in LC underwent degenerative changes characteristic of

apoptosis. On the other hand, the volume of LC reduced after RSD. Reduction of cell number in LC may be caused by apoptosis. The mechanisms underlying the neurodegeneration in the LC remain unclear. Several possibilities have been proposed for neurodegenerative damage in CNS, including oxidative stress (17-19). Our data showed that reduction of GSH content in the RSD group, indicating the existence of oxidative stress (data not shown). These data indicate that oxidative stress may cause degeneration of the LC as observed in CNS degenerative patients(20, 21). After treatment by melatonin, our results showed that an increase in neuronal number and volume of LC. Melatonin is a potent antioxidant in the CNS. Neuroprotective effect of melatonin has been reported in CNS damages such as neurodegenerative disease(22). Several mechanisms have been proposed for the neuroprotective effect of melatonin. Melatonin has been found to upregulate antioxidative defensive systems, including the activities of superoxide dismutase and glutathione peroxidase as well as levels of glutathione(23, 24). Furthermore, melatonin reportedly scavenges free radicals(25). Moreover, several studies have suggested that melatonin may upregulate GDNF mRNA levels(26, 27). On the other hand, one study has shown that an increased neuronal number in LC after REM sleep deprivation(28). In our study, there is a reduction of cell number in LC. Reduction of cell number in LC may be caused by apoptosis.

Conclusion:

Taken together, the present study demonstrates that reduction of cell number in the LC was accompanied by neurodegeneration, which is consistent with the other findings in the pathophysiology of sleep deprivation. Furthermore, apoptosis may be one of the mechanisms underlying the RSD in the LC. Systemic melatonin significantly protected LC neuronal population from cell death. These results indicate that melatonin may be therapeutic in the treatment of degeneration of the LC.

Conflict of Interest:

The authors declared no Conflict of Interests.

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