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Melatonin attenuates dexamethasone-induced spatial memory impairment and dexamethasone-induced reduction of synaptic protein expressions in the mouse brain

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ABSTRACT

Chronic stress or prolonged exposure to high levels of glucocorticoid induces neuropathological alterations, such as dendritic atrophy of hippocampal or cortical neurons. The chronic administration of high doses of dexamethasone (DEX), a synthetic glucocorticoid receptor agonist, impairs long-term memory and motor coordination, reduces body weight and induces mortality in mice. DEX is typically administered clinically for a prolonged period. Therefore, we are interested in studying the mechanism by which chronic DEX administration affects cognitive function. In this study, we attempted to explore whether chronic DEX administration alters the process of memory formation and to determine the mechanism underlying the detrimental effect of DEX. The results showed that mice treated with DEX for 21 consecutive days had significantly impaired spatial memory in the Morris Water Maze task. Mice treated with DEX had prolonged water maze performance latencies and spent less time in the target quadrant compared to the control group. Furthermore, DEX reduced brain-derived neurotrophic factor (BDNF), N-methyl-D-aspartate (NMDA) receptor subunit (NR2A/B), calcium/calmodulin-dependent protein kinase II (CaMKII) in both the prefrontal cortex and hippocampus and synaptophysin in the prefrontal cortex. We also investigated whether melatonin, a hormone synthesized in the pineal gland, could protect against DEX-induced changes in spatial memory and synaptic plasticity. The results showed that mice pretreated with melatonin prior to the DEX treatment had shorter escape latencies and remained in the target quadrant longer compared to the group only treated with DEX. Melatonin significantly prevented a DEX-induced reduction in the expression of NR2A/B, BDNF, CaMKII and synaptophysin. The results from the present study demonstrate that melatonin pretreatment prevents cognitive impairment caused by DEX. However, the precise mechanism by which melatonin affects cognitive function requires further investigation.

1. Introduction

Stressful events induce the release of adrenal stress hormones, including catecholamines and glucocorticoids. Both animal and human studies have shown that these hormones have profound negative effects on cognitive function (Lupien and McEwen, 1997; McEwen and Sapolsky, 1995; Roozendaal et al., 2006). The influence of activated glucocorticoids on oxidative stress-induced neuronal cell death has been investigated in vitro using hippocampal model systems (Behl et al., 1997). Elevated levels of endogenous glucocorticoids have been observed to damage multiple regions of the brain, particularly the hippocampus, which plays an important role in memory, mood and behavior. Chronic stress or prolonged exposure to high levels of glucocorticoids induces neuropathological alterations, such as dendritic atrophy in hippocampal and/or striatal neurons (Conrad et al., 2007). The chronic high-dose administration of dexamethasone (DEX), a potent glucocorticoid receptor agonist, impairs long-term memory and motor coordination, reduces body weight and induces mortality in mice (Danilczuk et al., 2006). In addition, DEX may cause pathological changes in the hippocampus and impair memory in rats (Luine et al., 1994) and humans (Keenan et al., 1996; Starkman and Schteingart, 1981; Starkman et al., 1992). DEX is typically

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administered clinically for a prolonged period; therefore, it is important to elucidate the mechanism by which chronic DEX administration affects cognitive function.

In this study, we explored the mechanism underlying the detrimental effect of DEX on cognition and on the expression of brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family; N-methyl-D-aspartate (NMDA) receptor subunit (NR2A/B); calcium/calmodulin-dependent protein kinase II(CaMKII), a major calcium-dependent signaling factor; and synaptophysin, a presynaptic neuronal marker, in the hippocampus and prefrontal cortex.

Melatonin, a hormone mainly synthesized in the pineal gland, exerts regulatory roles on seasonal and circadian rhythms (Hardeland, 2009; Zawilska et al., 2009). In addition, melatonin contributes to the protection of nuclear and mitochondrial DNA damage (Reiter et al., 2006) and is a highly effective direct free radical scavenger and indirect antioxidant (Tan et al., 2010). Melatonin affects cell membrane receptors, intracellular signaling cascades and neurotransmission and has antioxidant and anti-inflammatory effects (Cuzzocrea et al., 2004; Manda and Reiter, 2010). Melatonin influences hippocampal function, regulates the expression of cell adhesion molecules and serotonin release and facilitates short-term memory in rats. Melatonin also inhibits the pro-oxidant activity of aluminum in the rat hippocampus (Gomez et al., 2005). In the present study, we attempted to determine whether melatonin provides neuroprotection against DEX-induced changes in the levels of molecular markers of synaptic plasticity, such as BDNF, NR2A/B, CaMKII and synaptophysin, during the development of spatial memory in adult mice.

2. Materials and methods

2.1. Reagents and materials

DEX, melatonin and a mouse anti-β-actin monoclonal antibody were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Rabbit polyclonal antisera against NR2A/B and BDNF and mouse antisera against synaptophysin were purchased from Chemicon International, Inc. (Temecula, CA, USA). Mouse monoclonal antiserum against CaMKII was purchased from Santa Cruz Biotechnology, Inc. (California, USA). Biotinylated goat anti-rabbit IgG, normal goat serum, diaminobenzidine, and an ABC Elite kit were purchased from VectorLabs (Burlingame, CA, USA). An ECL Plus™ Western blotting kit was purchased from GE Health Care Bio-sciences AB (Uppsala, Sweden).

2.2. Experimental animals

All animal procedures were performed in compliance with Mahidol University's Code of Practice and the National Institutes of Health (USA) guidelines for care and use of laboratory animals. Male ICR mice (weighing 30–40 g) were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. The animal care committee of Mahidol University approved the experimental protocols. The experiments in the present study were designed to minimize the number of animals used and their suffering. Each mouse was housed individually in stainless steel wire cages with free access to food and water and maintained under standard conditions at 25 ± 2 °C and 60 ± 10% relative humidity with a 12 h light/dark cycle. All animals were handled daily for at least 1 week before initiating the experiments.

2.3. Treatment of the animals

Our previous study showed that 21 days of treatment with 60 mg/kg DEX was not lethal but significantly altered performance in the Morris Water Maze behavioral test (Tongjaroenbuangam et al., 2011). Therefore, a 60-mg/kg dose of DEX was used throughout this study. The mice were randomly assigned to the following treatment groups: control (Cont), DEX, melatonin (MEL) and MEL + DEX. All treatments were administered once daily at 6:00 pm, which coincided with the circadian peak of endogenous pineal and HPA secretory activity, for 21 consecutive days. Melatonin was solubilized in a vehicle (5% (v/v) ethanol in 0.9% saline). In the Cont group, the mice were intraperitoneally (i.p.) injected with the vehicle followed by 1 ml/kg of 0.9% saline. In the DEX group, the mice were treated with the vehicle followed by 60 mg/kg (i.p.) of DEX. In the MEL group, the mice were injected with 10 mg/kg of melatonin followed by 1 ml/kg (i.p.) of 0.9% saline. Finally, in the MEL + DEX group, the mice were injected with 10 mg/kg of melatonin followed by 60 mg/kg (i.p.) of DEX. The vehicle (5% (v/v) ethanol in 0.9% saline) or melatonin was administered 30 min before the 0.9% normal saline or DEX treatment.

2.4. Morris Water Maze task

After treatment for 21 consecutive days, the mice performed the Morris Water Maze task. The experiments were conducted in a circular pool (1.4 m in diameter and 60 cm deep) containing 28 °C water at 40 cm deep. The training and testing procedures were performed according to our previous study (Tongjaroenbuangam et al., 2011). Movements of the animals were analyzed using the Ethovision video tracking system, version 3.1 (Noldus Information Technology, the Netherlands).

2.5. Tissue preparation for 0.1% cresyl violet and immunohistochemistry staining

After the mice were treated with DEX for 21 days, 5–6 mice per group were deeply anesthetized with sodium pentobarbital (45 mg/kg i.p.), perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) and then perfused with 0.1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and post-fixed with the identical fixative overnight at 4 °C. Following cryoprotection in 30% sucrose in 0.1 M PBS overnight at 4 °C, free-floating coronal sections at the dorsal hippocampal level were cut into 25 μm thick sections using a cryostat microtome according to the stereotaxic atlas of the mouse brain (Paxinos and Franklin, 2001). The sections were maintained in 0.1 M PBS at 4 °C for 0.1% cresyl violet and immunoperoxidase staining and immunohistochemistry staining of BDNF and NR2A/B.

2.6. Cresyl violet staining and quantification of the neuronal CA3 hippocampal subfield

Dorsal hippocampus-containing sections from all intervals were mounted on glass slides and air-dried to assess the number of neuronal cells in the CA3 hippocampal subfield. Subsequently, the slides were soaked in a 0.1% cresyl violet working solution for 3 min followed by dehydration using an alcohol and xylene series. The slides were then coverslipped with Permount® (Fischer, USA). The number of cresyl violet positive neurons in the CA3 region was determined using a light microscope (at 20× magnification) fit with a digital camera. The image was displayed on a computer monitor, and cells with round, obvious nuclei and visible nucleoli were counted. Bilateral CA3 hippocampal regions were counted in 6–8 sections per mouse using a computerized image analysis system (Carl Zeiss, Germany). Counting was performed by one person who was unaware of the group identities. The values are expressed as the means ± SEM per section.