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Melatonin pretreatment prevented the effect of dexamethasone negative alterations on behavior and hippocampal neurogenesis in the mouse brain

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ABSTRACT

Glucocorticoids play various physiological functions via the glucocorticoid receptor (GR). Glucocorticoid is associated with the pathophysiology of depression. Dexamethasone (DEX), a synthetic GR agonist, has a greater affinity for GR than the mineralocorticoid receptor (MR) in the hippocampus of pigs and may mimic the effects of GR possession. DEX decreases neurogenesis and induces damage to hippocampal neurons that is associated with depressive-like behavior. Melatonin, a hormone mainly synthesized in the pineal gland, is a potent free radical scavenger and antioxidant. Melatonin alters noradrenergic transmission in depressed patients. It may be interesting to further explore the mechanism of melatonin that is associated with the role of stress as a key factor to precipitate depression and as a factor altering neurogenesis. In this study, we assessed the capability of melatonin to protect the hippocampus of mouse brains to counteract the effects of chronic DEX treatment for 21 days on depressive-like behavior and neurogenesis. Our results revealed that chronic administration of DEX induced depressive-like behavior and that this could be reversed by pretreatment with melatonin. Moreover, the number of 5-bromo-2-deoxyuridine (BrdU)-immunopositive cells and doublecortin (DCX; the neuronal-specific marker) protein levels were significantly reduced in the DEX-treated mice. Pretreatment with melatonin was found to renew BrdU and DCX expression in the dentate gyrus. Furthermore, pretreatment with melatonin prevented DEX-induced reductions in GR and an extracellular-signal-regulated kinase (ERK1/2) in the hippocampal area. Melatonin may protect hippocampal neurons from damage and reverse neurogenesis after chronic DEX by activating brain-derived neurotrophic (BDNF) and ERK1/2 cascades. These results revealed that melatonin pretreatment prevented the reduction of cell proliferation, immature neuron precursor cells, and GR and ERK1/2 expression. This finding indicates that melatonin attenuates the DEX-induced depressive-like behavior, supporting the notion that melatonin possesses anti-stress and neurogenic actions.

1. Introduction

Glucocorticoids (GCs) exert their functions via the glucocorticoid receptor (GR), influencing neuronal functions such as hippocampal long-term potentiation, depression [1–3] and cognitive function governed by the prefrontal cortex [4]. Furthermore, the GR has been revealed to regulate brain-derived neurotrophic factor (BDNF), stimulating neuronal cell death and changing the hippocampal neurogenesis of adult mice. Thus, it is also possible that the depressed brain shows abnormalities in GR function that contribute to structural changes [5]. In fact, deteriorated GR function has been suggested to underlie hypothalamic-pituitary adrenal (HPA) axis hyperactivity in depression, and GCs usually regulate the HPA axis via negative feedback inhibition, thus decreasing the

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production of GCs themselves. Thus, this effect is regarded to be mediated by the GR. Dexamethasone (DEX), a potent GR agonist, is more intensive in its affinity for GR than the mineralocorticoid receptor (MR) in the hippocampus of pigs and may mimic the effects of GR possession [6]. GCs are related to the pathophysiology of depression [3,7,8]. Depression was reported to decrease neurogenesis, suggesting that depression is related to a stress-induced reduction in the neurogenesis of the dentate gyrus of the hippocampus [9]. DEX is generally administered clinically for a prolonged period. In the present study, we investigated the possibility that chronic DEX-induced changes cause hippocampal dysregulation, including neurogenesis, GR, ERK1/2 and depressive behavior.

Melatonin, a hormone mainly synthesized in the pineal gland, exerts regulatory roles on seasonal and circadian rhythms [10,11] and acts directly as a highly effective free radical scavenger and indirectly as an antioxidant [12]. In addition, melatonin prevented the stress-induced reduction in cell proliferation of the dentate gyrus in maternally separated rats and decreased GR immunostaining [13]. Melatonin was shown to change noradrenergic transmission in depressed patients. Our previous study has shown that pretreatment with melatonin prior to DEX administration prevented DEX-induced reduction in BDNF protein expression in the hippocampus and prefrontal cortex area of adult mice [14]. In addition, depression was reported to decrease neurogenesis and alter BDNF, leading to hippocampal atrophy [15,16]. Furthermore, the pro-survival effect of melatonin on the hippocampal adult neurodevelopment has been reported [17]. This work widely demonstrated a key role for melatonin on hippocampal neurogenesis as well as its anti-depressant like effect also in the Porsolt's test. In the present study, we attempted to determine whether melatonin exerts an effect against DEX-induced changes in neurogenesis, GR and ERK1/2 during the development of depression in adult mice.

2. Materials and methods

2.1. Reagents and materials

DEX-21-phosphate disodium salt, melatonin and a mouse anti-β-actin monoclonal antibody were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Goat polyclonal antisera against doublecortin (DCX), mouse monoclonal antisera against GR, mouse antisera against ERK1/2 and p-ERK1/2 and biotinylated donkey anti-goat IgG were purchased from Santa Cruz Biotechnology Inc (California, USA). The 5-bromo-2-deoxyuridine (BrdU) immunohistochemistry kit (Calbiochem®) was purchased from Merck KGaA (Darmstadt, Germany). Biotinylated goat anti-rabbit IgG, normal donkey serum, diaminobenzidine and an ABC Elite kit were purchased from VectorLabs (Burlingame, CA, USA).

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 the use and care of laboratory animals. Male ICR mice (weighing 30–40 g; 8-week-old) were obtained from the National Laboratory Animal Center, Mahidol University, Thailand. The experimental protocols were approved by the Animal Care Committee of Mahidol University. All experiments were designed to minimize the number of animals used and their suffering. Each mouse was housed individually in a stainless steel wire cage 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 experiments.

2.3. Treatment of 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 [14]. Therefore, a 60 mg/kg dose of DEX was used throughout this study. Mice were randomly assigned to the following treatment groups: control (Cont), dexamethasone (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 vehicle (5% (v/v) ethanol in 0.9% saline). In the Cont group, the mice were intraperitoneally (i.p.) injected with vehicle followed by 1 ml/kg of 0.9% saline. In the DEX group, the mice were treated with vehicle followed by 60 mg/kg (i.p.) of DEX. Our previous study showed that 21 days of pretreatment with 10 mg/kg melatonin after DEX administration attenuates DEX-induced spatial memory impairment and reduction of synaptic protein expressions [14]. In addition, Crupi's group [18] found that the depressive-like state and the reduction in hippocampal cell proliferation caused by chronic corticosterone treatment was reversed by exogenous administration of melatonin at 8 mg/kg. Therefore, a 10 mg/kg dose of melatonin was used throughout this study. In the MEL group, the mice were injected with 10 mg/kg 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 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. Forced swimming test

The mice were treated with melatonin and/or DEX consecutively for 21 days. Then, after 24 h, performance in the forced swim test was assessed on the 22nd day after treatments began. Mice were moved from the animal house to the laboratory in their own cages and allowed to adapt to the laboratory conditions for 1–2 h. They were forced to swim in an open cylindrical container (diameter of 20 cm, height of 25 cm) containing 18 cm of water at 25 ± 1 °C. The total duration of immobility was assessed by a blind observer, who recorded the last 4 min of behavior in a test that lasted 6 min. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility was indicative of an antidepressant-like effect [19].

2.5. Western blot analysis

After 21 consecutive days of treatment, the mice were sacrificed, their brains were immediately removed, and the brain regions were dissected and maintained at −80 °C until the western blot analysis. Western blot analyses of DCX, GR, ERK1/2 and p-ERK1/2 protein levels were used to assess the ability of melatonin to protect adult mouse brains against the effects of DEX administration. Each dissected frozen hippocampus was homogenized in 3 ml of lysis buffer (20 mM Tris, pH 7.4; 150 mM NaCl; 1 mM Na3VO4; 10 mM NaF; 1 mM EDTA; 1 mM EGTA; 0.2 mM phenylmethylsulfonyl fluoride; 1% Triton X-100; 0.1% SDS; 0.5% deoxycholate) with a protease inhibitor and phosphatase inhibitor cocktail. The lysates were centrifuged at 12,000 × *g* for 15 min, and the supernatants were collected. The protein concentrations were quantified using the Lowry method [20] with bovine serum albumin as a standard protein. The protein samples (approximately 20–30 μg) were prepared from the collected supernatants and mixed with sample buffer (62.5 mM Tris–HCl, pH 6.8; 2% SDS; 10%