Melatonin ameliorates amygdala-dependent emotional memory deficits in Tg2576 mice by up-regulating the CREB/c-Fos pathway

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HIGHLIGHTS

- Melatonin improved amygdala-dependent emotional memory in Tg2576 mice.
- Melatonin increased the expression of c-fos protein levels in basolateral amygdala.
- Melatonin increased the expression of pCREB/CREB ratio in basolateral amygdala.
- CREB/c-Fos pathway play critical role for the efficacy of melatonin in Tg2576 mice.

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ABSTRACT

The effects of melatonin on spatial memory deficits in Alzheimer’s disease (AD) have been thoroughly investigated. Our previous study demonstrated that melatonin rescues hippocampus-dependent spatial memory deficits by arresting hippocampal pathological progression in an animal model of AD, which occurs via the inhibition of GSK3β and an increase in c-Fos. Based on the interaction between the amygdala and hippocampus, it is important to determine whether melatonin also improves amygdala-dependent emotional memory to understand the mechanism of melatonin amelioration of memory deficits in AD. The basolateral amygdala (BLA) is essential for the processing of emotions, including cued fear conditioning and anxiety. In the present study, we intraperitoneally injected Tg2576 mice with melatonin for 4 months and measured amygdala-dependent emotional memory using cued fear conditioning and a step-down passive avoidance test; the expression of c-Fos, Arc, phosphorylated CREB (pCREB) and other related genes were subsequently measured using Real-time polymerase chain reaction (RT-PCR) and Western blot in BLA. Our findings suggest that melatonin ameliorates amygdala-dependent emotional memory in AD via up-regulation of the pCREB/c-Fos pathway.

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1. Introduction

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by severe deficits in cognitive function. Abnormal hyperphosphorylation of microtubule associated protein tau (p-tau) and overproduction of β-amyloid (Aβ) are the 2 hallmarks in AD brains [1]. Disruptions in synaptic plasticity are associated with multiple neurodegenerative diseases, such as AD [2]. Synaptic plasticity and synaptic-related proteins comprise the major mechanisms that underlie learning and memory [3,4].

Many immediate early genes (IEGs) are linked to synaptic plasticity [5]. c-fos, which represents the first identified IEG [6], is indispensable for the maintenance of normal synaptic plasticity. Mice that lack c-Fos in the brain exhibit impairments in spatial reference and contextual learning, as well as decreased long-term potentiation (LTP) of synaptic transmission at CA3-to-CA1 synapses.
2. Materials and methods

2.1. Animals and treatments

Tg2576 mice harboring human APP 695 with the Swedish double mutation (HuAPP695; K670N/M671L, Jackson Lab) were intraperitoneally injected with 10 mg/kg melatonin (Sigma, St. Louis, MO, USA) in 0.9% saline or an equal volume of dimethylsulfoxide (DMSO) in 0.9% saline as a vehicle control once every other day for 4 months from 8 months old [22] (n = 20 per group, equal numbers of males and females). The body weight was monitored weekly. The genotypes of the mice were assessed via polymerase chain reaction (PCR) using tail clip digestions [33]. All mice were maintained at 24 ± 2 °C on a daily 12-h light-dark cycle with ad libitum access to food and water; all behavioral procedures were performed during the light cycle. The mice were sacrificed under deep anesthesia following the behavioral experiments. The tissues were collected for WB and RT-PCR analyses. The animal experiments were conducted according to the “Policies on the Use of Animals and Humans in Neuroscience Research” approved by the Society for Neuroscience in 1995 and were supervised by the Animal Administration and Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology [22].

2.2. Cued fear conditioning and fear expression

To determine the effect of amygdala-dependent emotional memory, cued fear conditioning [27] was implemented. The mice underwent cued fear conditioning and were tested for fear expression [4,29,34,35]. The mice were trained and tested in a sound attenuating chamber with a gridding floor that could administer an electric shock, an overhead control apparatus that consisted of a video camera, and an acoustic generator in one corner of the chamber. Freezing was defined as a complete absence of movement. Movement was recorded by the camera. In the cued fear conditioning training phase, the mice were placed in the chamber with no interference for 120 s; a 30-s 80-dB white noise (conditioned stimulus CS) was subsequently presented, and a 0.5 mA foot shock (unconditioned stimulus US) was administered for 2 s prior to the end of the noise. The CS–US pair was presented three times, with a 1-min inter-trial interval after each stimulus was completed. The cued test was performed in a different context; the mice were placed in the chamber and heard an auditory CS for 30 s after a 3-min undisturbed period without a stimulus; the freezing time was then recorded continuously for 3 min. The chamber included a Plexiglas front and gray side and back walls, and the chamber floors consisted of 26 stainless steel rods. The rods were connected to a shock generator via a cable harness.

2.3. Step-down passive avoidance test

The inhibitory avoidance apparatus was a 50 × 25 × 25 cm³ acrylic box with a floor that consisted of a grid of parallel stainless steel bars (1 mm diameter) spaced 1 cm apart. A platform that was 10 cm² and 2 cm high was placed in the center of the floor (Institute of Materia Medica). An electric current (40 V) was delivered to the grid floor using an isolated stimulator. In the training session, immediately after the mouse stepped down on the grid, it received a scrambled foot shock (0.3 mA, 2 s). During the test session, each mouse was placed on the platform, and its latency to step down (step-down latency, SDL) on the grid with all four paws was measured with an automatic device; the number of errors in 3 min (CE) was also measured. A retention test was conducted 24 h after the first training session. The SDL and CE were recorded to measure long-term memory. The cut-off time was 180 s [36,37].

2.4. RT-PCR

Total RNA was isolated using Trizol reagents according to the instructions (Tiangen Technologies, Beijing, China). Total RNA (3 μg in 25 μL) was reverse transcribed using a commercial kit (Takara, Dalian, China), and the produced cDNA (1 μL) was used to detect the transcripts. The following primers were used: c-fos, 5′-CCGAAGGGAACGGAATAAG-3′ (forward primer), 5′-TCTGGAGCAAGTCAT-3′ (reverse primer).
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