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The effect of Eleutheroside E on behavioral alterations in murine sleep deprivation stress model

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ABSTRACT

Eleutheroside E (EE), a principal component of *Eleutherococcus senticosus*, has been reported to have anti-inflammatory and protective effects in ischemia heart etc. However, whether it can mitigate behavioral alterations induced by sleep deprivation, has not yet been elucidated. Numerous studies have demonstrated that memory deficits induced by sleep deprivation in experimental animals can be used as a model of behavioral alterations. The present study investigated the effect of EE, on cognitive performances and biochemical parameters of sleep-deprived mice. Animals were repeatedly treated with saline, 10 or 50 mg/kg EE and sleep-deprived for 72 h by the multiple platform method. Briefly, groups of 5–6 mice were placed in water tanks (45×34×17 cm), containing 12 platforms (3 cm in diameter) each, surrounded by water up to 1 cm beneath the surface or kept in their home cage. After sleep deprivation, mice showed significant behavioral impairment as evident by reduced latency entering into a dark chamber, locomotion and correctly rate in Y maze, and increased monoamines in hippocampus. However, repeated treatment with EE restored these behavioral and biochemical alterations in mice. In conclusion, the beneficial effect of EE may provide an effective and powerful strategy to alleviate behavioral alterations induced by sleep deprivation.

1. Introduction

Insomnia often results in symptoms related to central nervous system (CNS) function including reduced activity and muscle endurance and impaired coordination, concentration, attention, learning and memory (Afari and Buchwald, 2003; Chen et al., 2009). In modern life, sleeplessness has come to be recognized as a serious source of many chronic illnesses that can significantly impair a person's functioning and have a negative impact on their quality of life. Persistent sleeplessness gives rise to brain dysfunction (also called central fatigue) which is thought to be the sensation to decline behavioral motivation and to prevent injury of a body part well before it lapses into a serious state. In clinical terms, it is defined as difficulty in the initiation of, or the ability to sustain, voluntary activities. Therefore, central fatigue represents a failure to complete mental tasks that require self-motivation and internal cues in the absence of demonstrable cognitive failure or motor weakness (Chaudhuri and Behan, 2000, 2004).

Previous studies have provided evidence for the involvement of monoamines in fatigue induced by sleep deprivation (Foley et al., 2006). Newsholme et al. were the first to hypothesize that increased concentrations of brain 5-HT a system known to regulate cognition and behavior (Struder and Weicker, 2001) could alter CNS function, thereby causing distorted perception of fatigue (Newsholme et al., 1987). Recent work has focused on the monoamines serotonin and dopamine in the mechanisms of sleep deprivation. Studies in humans and animals support a role of the alteration of 5-HT, dopamine in the brain in central fatigue (Bailey et al., 1992, 1993; Davis et al., 2003; Foley et al., 2006); the contribution of increased oxidative stress in the brain leading to mental fatigue is also well documented. Studies have verified that memory deficits induced by sleep deprivation are accompanied by an increase in hippocampus oxidative indices in mice (Silva et al., 2004).

Eleutheroside E (EE), the principal active constituent of *Eleutherococcus senticosus* (*Acanthopanax senticosus*), is known to have anti-inflammatory effect by suppressing the gene expression of inflammatory proteins through inhibiting NF-KAPPA B and AP-1 binding activities (Tokiwa et al., 2006; Yamazaki et al., 2006, 2007) and protective effects in ischemia heart after acute myocardial infarction induced by pituitrin. In addition, it has also been reported to reduce physical fatigue and enhance physical strength in chronic swimming

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stressed rats (Nishibe et al., 1990). However, as far as we known the effects of EE on behavioral alterations induced by sleep deprivation have not yet been studied. To explore this, we have used the multiple platforms method (Bjorvatn et al., 2002; Hu et al., 2003; Lopez-Rodriguez et al., 2003; Silva et al., 2007, 2004; Youngblood et al., 1997) in which the extent of fatigue has been evaluated by the changes in neurocognitive behavior.

Therefore, the purpose of the present study was to explore the effectiveness of EE on behavioral changes induced by 72 h sleep deprivation, such as passive avoidance, locomotor activity, and Y maze. Biochemical estimations were also carried out to establish whether the antioxidant potential of this compound was responsible for this activity. We also conducted preliminary experiments to investigate the influence of EE treatment on hippocampus neurotransmitter levels.

2. Materials and methods

2.1. Animals

Five-week-old male ICR mice (weighing 18–22 g) were used and housed under automatically controlled conditions of temperature (25 ± 1) °C with 60% relative humidity and had free access to laboratory standard diet and water. The room lights were on for 12 h/day starting at 7:00h. The care and treatment of experimental animals conformed to the guidelines for the ethical treatment of laboratory animals.

2.2. Materials

Serotonin and dopamine ELISA kits were purchased from R&D, USA, while reduced glutathione (GSH), malondialdehyde (MDA) and protein quantization measurement kits were purchased from Nanjing Jiancheng Bioengineering Institute. Eleutheroside E (with its purity of 95%) was isolated from *E. senticosus* by our laboratory (shown in Fig. 1). Other reagents were obtained from usual commercial sources.

2.3. Drug administration

EE dissolved in saline solution, was administered for 10 days, 1 h before each behavioral test during the 72 h sleep deprivation experiments and saline was used as the control solution. Both substances were given intragastrically at a volume of 10 ml/kg of body weight. The animals were divided into four groups and received saline and EE at 10 or 50 mg/kg body weight.

2.4. Sleep deprivation

Sleep deprivation was conducted using the modified multiple platform method, as described previously (Silva et al., 2007, 2004; Suchecki and Tufik, 2000). Briefly, groups of 5–6 mice were placed in water tanks (45 × 34 × 17 cm), containing 12 platforms (3 cm in diameter) each, surrounded by water up to 1 cm beneath the surface, for 72 h. In each tank, mice were coming from the same cage where they were previously housed, and were capable of moving inside and jumping from one platform to the other. Food and water were provided ad libitum throughout the study and in addition, the water in the tanks was changed daily.

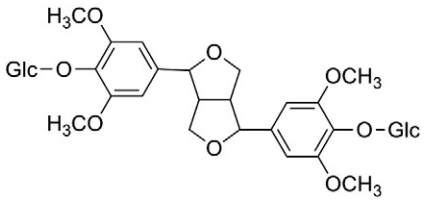

Fig. 1. The chemical structure of Eleutheroside E isolated from *Eleutherococcus senticosus*.

2.5. Experimental procedure

Animals were divided in four groups each comprising of twelve animals. General procedures included previous treatment with saline or EE (10 or 50 mg/kg) during 10 days with or without sleep deprivation. After drug or saline administration for 7 days, animals were submitted to 72 h sleep deprivation or remained in their home cages and acted as the control. Behavioral tests were performed after every 24 h sleep deprivation and each experimental group was further divided into two groups (n = 6 each). Half of these animals were tested for Y-maze, while the others were subjected to passive avoidance test and locomotor activity. Animal weights were recorded before (day 7) and after sleep deprivation (day 10).

2.6. Hippocampus dissection and homogenate

After 72 h sleep deprivation, the animals were killed by decapitation and the brain was removed immediately and washed with ice-cold saline. Then, the hippocampus was quickly dissected, weighed and frozen at −80 °C for biochemical analysis. The whole procedure was completed within 2 min and brain tissue was maintained on ice throughout the experimental procedure. A 10% (w/v) tissue homogenate was prepared in normal saline which was used for biochemical assays.

2.7. Behavioral tests

2.7.1. Passive Avoidance Task

The test was carried out essentially as described by de Oliveira et al. (2004), and Silva et al. (2007, 2004). The apparatus employed was a two-way shuttle-box intercommunicated by a guillotine door placed between the modular testing chambers. One chamber was clear, while the other remained in the dark. In the training session on the day before sleep deprivation (day 7), animals were given a foot shock (0.5 mA) whenever they entered the dark compartment. The training session ended after the animal stayed in the clear chamber for more than 300 s (Hong Wang et al., 2003). In the test sessions, the mice were again placed in the illuminated chamber, but no foot shock was applied. And latency to enter through the chambers was registered in each session.

2.7.2. Measurement of locomotor activity

Locomotor activity was assessed in a digital photoactometer, which consisted of six (33 × 10 × 11 cm) cages that were surrounded with photoelectrical horizontal detection sensors. The total number of counts indicating movement of the mice was measured for 30 min and was expressed as total photocell counts of the photoactometer for 30 min per animal. Modification of locomotor function was measured in all animals on the day before sleep deprivation (day 7) and after sleep deprivation for 24 h, 48 h, and 72 h respectively.

2.7.3. The Y-maze test

The Y maze consisted of three equal arms (40 × 15 × 15 cm) with a stainless-steel grid floor. The work current intensity was 0.8 mA and while the running voltage was 42 V. Three arms were randomly designated as the start arm. After a 2-min habituation in it, the mice were given a foot shock, which forces the animals to escape to the left arm (correct, no foot shock) and the entry into the right arm (error) was punished by a further foot shock. In the training session, each mouse was given trials until the correct ratio was reached within 90% in 5 min, otherwise the mice were abandoned. In the testing sessions, the mice were placed back in the same starting arm, and were given