Brain Research Bulletin 121 (2016) 105–114

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Evaluation of adaptogenic-like property of methyl jasmonate in mice exposed to unpredictable chronic mild stress

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ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form
16 November 2015
Accepted 18 November 2015
Available online 22 November 2015

Keywords:
Methyl jasmonate
Unpredictable chronic mild stress
Corticosterone
Oxidative stress
Neuroprotection
Adaptogen

ABSTRACT

This study was undertaken to evaluate the adaptogenic-like activity of methyl jasmonate (MJ) in mice exposed to unpredictable chronic mild stress (UCMS). Male Swiss mice were treated with MJ (25–100 mg/kg, i.p.) 30 min before exposure to UCMS daily for 14 days prior to testing for memory and anxiety. Thereafter, the blood glucose and serum corticosterone levels were estimated using glucometer and ELISA. The brain concentrations of malondialdehyde (MDA) and glutathione (GSH) were estimated using spectrophotometer. Brain histology and the population of healthy neurons in the hippocampal regions were also assessed. MJ reversed anxiety and memory impairment produced by UCMS, which suggest adaptogenic-like property. The reduction in the weight of adrenal gland and liver in MJ-treated groups further indicates adaptogenic activity. It further decreases the blood glucose and serum corticosterone levels in UCMS-mice. Also, MJ decreases the concentrations of MDA and elevated the levels of GSH in the brain of mice exposed to UCMS. Brain histology revealed that MJ attenuated UCMS-induced degeneration and death of neuronal cells in the pyramidal layer of the cornu ammonis 3 (CA3) and the sub-granular zone of the dentate gyrus of the hippocampus. Moreover, MJ decreased the population of dead neuronal cells of the pyramidal layer of the CA3 and the sub-granular zone of the dentate gyrus of the UCMS-mice, which suggests neuroprotection. Taken together, these findings suggest that MJ demonstrated adaptogenic-like activity in mice; which might be related to modulation of serum corticosterone levels, inhibition of oxidative stress and neuroprotection.

1. Introduction

Adaptogens were once defined as substances that enhance the "state of non-specific resistance" of an organism against stress (Panossian, 2013; Brekhman and Dardymov, 1969). However, Panossian et al., 1999 referred to adaptogens as "new class of metabolic regulators which increase the ability of an organism to adapt to environmental factors and to avoid damage from such factors". Thus, adaptogens help to normalize body functions and strengthen body systems compromised by stress. Adaptogens also offer protection against environmental assaults and emotional trauma (Panossian and Wikman, 2010). A number of clinical trials have shown that adaptogens possess anti-fatigue/anti-stress property and increased both mental and physical performances, particularly in preventing mental exhaustion (Panossian and Wikman, 2010). Studies on animals and isolated cells revealed that adaptogens exhibited neuroprotective, anti-fatigue, anti-depressive, anxiolytic and nootropic effects (Panossian and Wagner, 2005; Panossian and Wikman, 2010).

The stress-protective effect of adaptogens has been linked with the regulation of homeostasis via several mechanisms that are associated with the hypothalamic–pituitary–adrenal (HPA) axis and key mediators of stress responses (Panossian et al., 2007; Wiegant et al., 2008; Panossian and Wikman, 2010). The beneficial effects of adaptogens have also been linked to their capacity to inhibit the formation of free radicals and to modulate other processes involved in the adaptation of the organism to stress (Wiegant et al., 2008; Panossian and Wikman, 2010). Moreover, adaptogens are generally believed to boost energy or resilience in the face of stress and to enhance the defense mechanisms of the body (Panossian,

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http://dx.doi.org/10.1016/j.brainresbull.2015.11.016
0361-9230/

2013; Bucci, 2000; Ellis and Reddy, 2002). A number of medicinal plants such as *Rhodiola rose,* Asian ginseng (*Panax ginseng), Ginkgo biloba, Ocimum sanctum, Withania somnifera*, Siberian ginseng (*Eleutherococcus senticosus)*, *Schizandra chinensis, R. rosea* and *Bryonia alba* have been shown to possess adaptogenic property and some of them are available commercially for stress management (Panossian, 2013; Panossian et al., 2012; Ellis and Reddy, 2002). Clinically, adaptogens have been used to prevent fatigue, memory deterioration, anorexia, insomnia, mood disorders and to aid recovery from debilitating diseases (Panossian, 2013; Rege et al., 1999).

Methyl jasmonate (MJ) is an anti-stress plant hormone that was first isolated from *Jasminum grandiflorum* but now obtained through pharmaceutical synthesis (Fingrut and Flescher, 2005; Bowles, 1990). MJ and its congeners are widely used in aromatherapy for treatment of depression, nervousness and memory dysfunctions (Kuroda and Inoue, 2005). MJ plays a key role in the defense of plant against external stressors through activation of the adaptive mechanisms of the plant cells (Cesari et al., 2014; Fingrut and Flescher, 2005; Bowles, 1990). Specifically, the beneficial effect of MJ in stress is related to the formation of proteinase inhibitor (PI) proteins, which are array of defensive chemical substances that protect plants against stressors (Cesari et al., 2014; Bowles, 1990). The exposure of plants to stressors triggers the formation of jasmonates, which in turns activates PI gene expression (Fingrut and Flescher, 2005; Bowles, 1990). The rapid rise in the levels of MJ in plants exposed to chronic damaging adverse situations further confirm its role in mitigation of stress (Cesari et al., 2014; Rotem et al., 2005). The adaptive effect of MJ in plants against various stressors suggests that it might also aid adaptation to stress in animals and may also explain its usefulness in nervousness in humans. Thus, this study was designed to evaluate the adaptogenic-like property of MJ in mice exposed to UCMS, an animal model that closely mimic the ways humans encounter stressors on daily basis.

2. Materials and methods

2.1. Experimental animals

Male Swiss mice (20–25 g; 7 weeks old) obtained from the Central Animal House, University of Ibadan, were used in the study. They were housed in plastic cages at room temperature with a 12 h light/dark cycle and were fed with rodent pellet diet and water ad libitum. All experimental procedures in this study were performed in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). All efforts were made to minimize animal sufferings and were scarified under ether anesthesia.

2.2. Preparation of MJ

MJ obtained from Sigma–Aldrich, Germany, was prepared according to the procedure previously described by Umukoro et al., 2011. Briefly, MJ was dissolved in 95% ethanol and this solution was further diluted with distilled water. The final concentration of ethanol in the solution used for the study did not exceed 1%. The doses of MJ used in this study were selected based on the results obtained from previous investigations (Umukoro et al., 2011).

2.3. Unpredictable chronic mild stress (UCMS) paradigm

The UCMS protocol was carried out in accordance with the method previously described by Yalcin et al., (2005) with slight modifications. After 2 weeks of acclimatization, mice were assigned to different experimental groups ($n = 6$) in a semi-randomized fashion, so that mean body weights were comparable in all groups.

Table 1
Schedule of stressors in unpredictable chronic mild stress paradigm in mice.

Days	Stressors and duration
1	Forced swim (5 min); food deprivation (12 h)
2	Damp sawdust (120 min); hypoxia (15 min)
3	Tail pinch (5 min); sawdust free cage (90 min)
4	Food deprivation (12 h); change of cage mates(30 min)
5	45° cage tilting (120 min); forced swim (5 min)
6	Food deprivation (12 h); hypoxia (15 min)
7	Exposure to predator odor (30 min); water deprivation (12 h)
8	45° cage tilting (180 min); forced swim (6 min)
9	Sawdust free cage + 200 ml water (120 min); tail pinch (5 min)
10	Hypoxia (15 min); water deprivation (12 h)
11	Damp sawdust (120 min); tail suspension (5 min)
12	Forced swim (5 min); food deprivation (12 h)
13	Exposure to cat meowing (30 min); fawdust free cage (120 min)
14	Social defeat (5 min), food and water deprivation (12 h)

Thereafter, mice received i.p injection of MJ (25, 50 and 100 mg/kg) or vehicle (1% ethanol, 10 ml/kg; which served as chronic stress group) 30 min before exposure to different stressors in the UCMS paradigm (Table 1). This procedure was repeated daily for 14 consecutive days and the stressors were applied in a random order and at varying times to maximize unpredictability. Thereafter, behavioral tests for memory and anxiety were carried out using validated behavioral procedures. The non-stress control group also received the vehicle (1% ethanol, 10 ml/kg) but was not exposed to stressors in the UCMS paradigm.

2.3.1. Test for memory performance using Y-maze paradigm

The effect of MJ on memory in mice exposed to UCMS was assessed using the *Y*-maze paradigm as previously described (Yan et al., 2001). Chronic stress mice and non-stress counterparts were placed individually at arm A of the *Y*-maze and allowed to explore all the three arms freely for 5 min. The number and sequence of arm entries were recorded and the apparatus was cleaned after each test. An entry was scored when the four paws of the animals were completely in the arm of the *Y*-maze. The percentage alternation, which is a measure of memory function, was calculated by dividing the total number of alternations by the total number of arm entries, subtracted by two, and then multiplied by 100 (Yan et al., 2001). An alternation behavior was defined as consecutive entries into all three arms (i.e ABC, CAB or BCA but not BAB) (Yan et al., 2001).

2.3.2. Light/dark (L/D) transition test for anxiety

The L/D transition test was employed to assess the effect of MJ on chronic stress-induced anxiety in mice according to the procedure described by Li and Quock, 2001. The animals were placed individually at the center of the bright compartment and were observed for a period of 5 min. The parameters measured were the duration of time spent in each of the L/D compartments. Ethanol (10%) solution was used to clean the box after each test to prevent odor bias.

2.3.3. Elevated plus-maze (EPM) test for anxiety

The EPM test was further used to assess the effect of MJ on chronic stress-induced anxiety in mice according to the procedure described by Lister, (1987). The animals were placed individually at the center of the maze with its head facing an open arm and allowed to explore the maze for 5 min. The parameters measured were the frequency and duration of arm entries. An entry was scored when the four paws of the animals were completely into one arm of the EPM. Ethanol solution (10%) was used to clean the plus maze after each test. The results were expressed as time spent in arms and percentage of number of entries in arms (mean ratio of entries in an arm to total entries in both open and closed arms).