Neuroscience Letters 585 (2015) 54–59

Contents lists available at ScienceDirect

Neuroscience Letters

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

Short communication

3,4,5-Trimethoxycinnamin acid ameliorates restraint stress-induced anxiety and depression

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HIGHLIGHTS

- A pharmacological activity of TMCA (trimethoxy cinnamic acid) is measured for anti-stress and anti-depressant.
- In parallel with behavioral data, long-lasting stimulation of ΔFosB in nucleus accumbens shell subregion was exhibited in response to TMCA.
- Reduced SC1, the candidate of ΔFosB target gene, mRNA expression in nucleus accumbens of mice administrated by restraint stress was profoundly reversed by TMCA.
- Collectively, TMCA treatment may be a therapeutic strategy for anxiety and depression.

ARTICLE INFO

Article history:
Received 18 October 2013
Received in revised form 24 October 2014
Accepted 12 November 2014
Available online 14 November 2014

Keywords:
TMCA
Anxiety
Depression
ΔFosB
SC1

ABSTRACT

The present study investigated whether 3,4,5-trimethoxycinnamic acid (TMCA) treatment ameliorated restraint stress-provoked anxiety- and depression-like behaviors in mice. Fourteen consecutive days of restraint stress produced anxiety- and depression-like behaviors, including reduced time and frequency in the open arms of the elevated plus maze (EPM), as well as enhanced immobility times in the forced swim test (FST). However, TMCA (50 mg/kg) treatment ameliorated this effect; mice showed increased time and frequency of visits in the open arms of the EPM and showed reduced immobility in the FST. In parallel with the behavioral data, long-lasting stimulation of ΔFosB in the nucleus accumbens shell subregion was exhibited in response to TMCA. Furthermore, a reduction in expression of SC1, a target of ΔFosB, in the nucleus accumbens of mice subjected to restraint stress was significantly reversed by TMCA (50 mg/kg) treatment. Collectively, these results suggest that TMCA treatment might provide a therapeutic strategy for treatment of anxiety and depression.

1. Introduction

Chronic stress is a well-known risk factor of psychiatric illness, including anxiety and depression. Normal adaptation in response to stressful stimuli is necessary for survival, whereas abnormal adaptation, such as persistent and excessive activation of the hypothalamic–pituitary–adrenal axis or activation of the mesolimbic dopamine pathway, can lead to psychiatric disorders [2,13,17,25,30]. The mesolimbic dopaminergic system modulates reward and motivation [7]. The nucleus accumbens (NAc) is a key region in the brain's reward region and is closely linked to controlling the stress response [4,8,10,21]. The alteration of several proteins, such as delta-fosB (ΔFosB) and brain-derived neurotrophic factor (BDNF), within the brain's reward circuits reportedly play an important role in the expression of depressive-like behavior in response to stressful situations, including social defeat and restraint stress [2,21,29]. In particular, ΔFosB, a truncated splice variant of FosB, is induced in the NAc by social defeat, as well as acute and chronic restraint stress [20,21,27,29]. Furthermore, Vialou et al. suggested that ΔFosB induction in the NAc was a key molecular determinant of resilience or susceptibility to a stressful situation and was required for antidepressant action; mice treated with fluoxetine had significantly increased levels of ΔFosB during chronic social defeat, concomitant with enhanced social interaction time [29]. According to previous studies, ΔFosB

Abbreviations: ΔFosB, delta FBJ murine osteosarcoma viral oncogene homolog B; BDNF, brain-derived neurotrophic factor; EPM, elevated plus maze; FST, forced swimming test; HPA, hypothalamic–pituitary–adrenal axis; NAc, nucleus accumbens; PB, pentobarbital-induced; TCMA, 3,4,5-Trimethoxycinnamic acid.

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http://dx.doi.org/10.1016/j.neulet.2014.11.016
0304-3940/

induction in the NAc is thought to be an important event regulating mood disorders, such as depression, in response to stressful stimuli. Recently, natural products have shown promise as new anti-depressant agents that possess high efficacy and fewer side effects.

3,4,5-Trimethoxycinnamic acid (TMCA) is a bioactive component derived from Onji, the roots of *Polygala tenuifolia*. Several studies have suggested that TMCA has anti-stress and sedative effects. Intraperitoneal injection of TMCA ameliorated the shortened pentobarbital-induced sleeping time of rats exposed to repeated cold stress and corticotrophin-releasing hormone-induced stress [11]. In addition, several extracts from *P. tenuifolia* enhance the anti-depressive effects of norepinephrine reuptake inhibitors by suppressing transport [5]. These studies suggest that TMCA may have potential anxiolytic and anti-depressive actions. In the current study, we showed that TMCA treatment ameliorates anxiety- and depression-like behaviors from 14 consecutive days of restraint stress, concomitant with enhanced ΔFosB protein and SC1 mRNA expression in the NAc.

2. Materials and methods

2.1. Experimental agent and mice

All animal experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee at Ewha Women's University. Male 7-week-old C57BL/6J mice were obtained from Daehan Biolink, Inc., (Eumsung, Chungbuk, Korea). Mice were housed in clear plastic cages under a light–dark cycle (lights on at 06:00 and off at 18:00 h). Mice were given a standard irradiated chow diet (Purina Mills, Seoul, Korea) *ad libitum* and were maintained in a specified pathogen-free state. TMCA was purchased from Sigma and Yuhan Pharm. (Seoul, Korea).

2.2. Experimental design

To examine the effects of TMCA on anxiety- and depression-like behaviors induced by restraint stress, mice were divided into four groups ($n = 12$ per group): control, restraint stress, and restraint stress treated with TMCA (25 mg/kg or 50 mg/kg) (Fig. 1A). To induce restraint stress, mice were individually placed into a well-ventilated 50 mL conical tube, plugged with a middle tube 3 cm in length, and then the tube was capped. Mice were not able to move forward or backward in this device. Restraint stress was delivered to mice at set times daily from 11:00 AM to 1:00 PM for 2 h. Control mice remained undisturbed in their original cages. This procedure was repeated for 14 days unless otherwise indicated. After the exposure to restraint stress, mice were removed from the tube and returned to their original cage. To identify and adopt the set time that enhanced immobility time relative to control mice in the forced swimming test (FST), we measured immobility in time-dependent manner, on day 2, 9, and 15 after the last restraint stress exposure. Each group was independently assigned to time-dependent experiments to avoid variables such as increased stress due to repeated FST. TMCA dissolved in saline was administered per orally 1 h before restraint stress administration. Mice were only treated with TMCA during the 14 consecutive days of restraint stress administration.

2.3. Behavioral tests

To measure the degree of anxiety-like behavior, the elevated plus maze (EPM) test was conducted using an apparatus consisting of four arms (30 × 7 cm) made of black Plexiglas. The apparatus was elevated 50 cm above the ground. Two of the arms had 20-cm-high walls (enclosed arms) and the others had no walls (open arms).

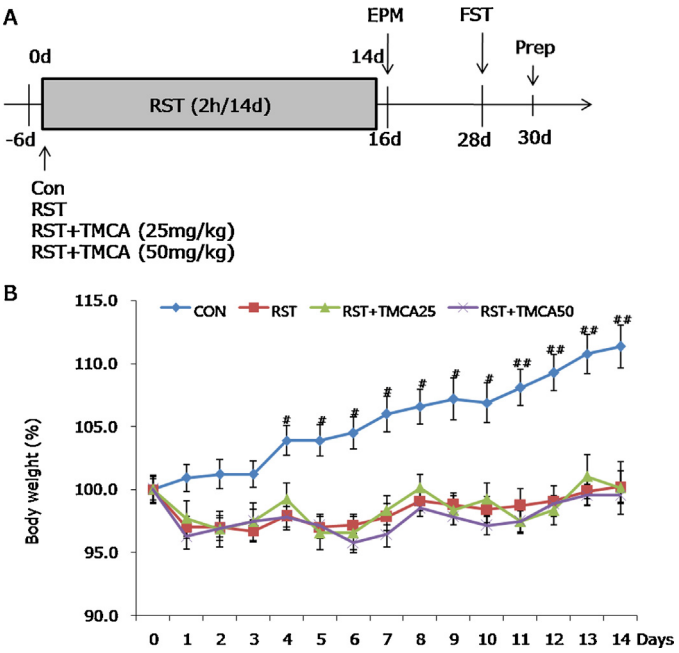

Fig. 1. TMCA treatment did not change body weights of mice exposed to 14 consecutive days of restraint stress. (A) A schematic representation of the experimental procedure. (B) The change in body weights of mice exposed to restraint stress. The data are presented as the mean ± SEM. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* day 0, respectively.

Mice were initially placed at the center of the platform and left to explore the arms for 5 min. The number of entries and the time spent in the open arms was scored.

To measure degree of depression-like behavior, the FST was conducted using a Plexiglas cylinder (height = 27 cm, diameter = 15 cm) containing water at a depth of 14–16 cm). Mice were subjected to pre-swimming for 15 min. One day later, mice were forced to swim for 6 min and immobility time was recorded [12].

2.4. Western blot analysis

Coronal sections of the whole brain (400 μm thickness) were taken and the NAc (bregma = 1.94–0.98) was extracted using micropuncture (diameter = 2 mm).

Protein samples (20 μg per lane) were separated on a 10% polyacrylamide gel by electrophoresis and transferred to a nitrocellulose membrane (Amersham Biosciences, Buckinghamshire, UK). Membranes were incubated with anti-FosB antibody (SC-48, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in blocking buffer at 4 °C overnight, washed, and incubated with a horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. The optical density of each band was measured using SCION (NIH Image Engineering).

2.5. Immunohistochemistry

Mice were perfused through the left cardiac ventricle with 10 mL of 10 mM phosphate buffer (pH 7.4) followed by 40 mL of a cold fixative (4% paraformaldehyde in 100 mM phosphate buffer). After perfusion, the brain was quickly removed, post-fixed for 18 h in the same fixative at 4 °C, and transferred to 10%, then 20%, and then 30% sucrose solution. Sections (40 μm thickness) were prepared using a vibratome (Leica, Wetzlar, Germany).

Free-floating sections were treated with 0.3% Triton-X100 for 30 min, and non-specific protein binding was blocked by incubation with 10% normal serum in PBS for 1 h. Sections were incubated