Progress in Neuro-Psychopharmacology & Biological Psychiatry 65 (2016) 1–16

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Age-dependent effects of esculetin on mood-related behavior and cognition from stressed mice are associated with restoring brain antioxidant status☆

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ARTICLE INFO

Article history:
Received 5 July 2015
Received in revised form 6 August 2015
Accepted 12 August 2015
Available online 18 August 2015

Keywords:
Age
Anti-depressive
Esculetin
Oxidative stress

ABSTRACT

Dietary antioxidants might exert an important role in the aging process by relieving oxidative damage, a likely cause of age-associated brain dysfunctions.

This study aims to investigate the influence of esculetin (6,7-dihydroxycoumarin), a naturally occurring antioxidant in the diet, on mood-related behaviors and cognitive function and its relation with age and brain oxidative damage. Behavioral tests were employed in 11-, 17- and 22-month-old male C57BL/6J mice upon an oral 35 day-esculetin treatment (25 mg/kg). Activity of antioxidant enzymes, GSH and GSSG levels, GSH/GSSG ratio, and mitochondrial function were analyzed in brain cortex at the end of treatment in order to assess the oxidative status related to mouse behavior.

Esculetin treatment attenuated the increased immobility time and enhanced the diminished climbing time in the forced swim task elicited by acute restraint stress (ARS) in the 11- and 17-month-old mice versus their counterpart controls. Furthermore, ARS caused an impairment of contextual memory in the step-through passive avoidance both in mature adult and aged mice which was partially reversed by esculetin only in the 11-month-old mice. Esculetin was effective to prevent the ARS-induced oxidative stress mostly in mature adult mice by restoring antioxidant enzyme activities, augmenting the GSH/GSSG ratio and increasing cytochrome c oxidase (COX) activity in cortex.

Modulation of the mood-related behavior and cognitive function upon esculetin treatment in a mouse model of ARS depends on age and is partly due to the enhancement of redox status and levels of COX activity in cortex.

1. Introduction

Oxidative damage is considered to be a likely cause of age-associated brain dysfunctions such as neuropsychiatric disorders and cognitive impairment (Pandya et al., 2013). In humans, the incidence of depression and impairment of cognitive function is higher among the elderly. Particularly, reactive oxygen species have been shown to modulate levels and activity of neurotransmitter systems (Dabrowiecki et al., 1985) involved in the neurobiology of depression (Cryan et al., 2002). Substantial data from animal and human studies support, on one hand, that major depression disorders (MDD) seem to be accompanied by an impairment of the total antioxidant status (Siwek et al., 2013) and, on the other hand, that antidepressant treatments may reduce oxidative stress (Behr et al., 2012). These studies suggest that augmentation of antioxidant defenses may be one of the mechanisms underlying the neuroprotective effects of antidepressants in the treatment of MDD (Xu et al., 2014). For instance, N-acetyl-cysteine has been shown to have a significant benefit on depressive symptoms in a randomized placebo-controlled trial (Berk et al., 2014). Additionally, curcumin has been shown to strongly interfere with neuronal redox homeostasis in the central nervous system (CNS) and to possess antidepressant activity in various animal models of depression (Zhang et al., 2014) and in humans (Lopresti et al., 2014). Furthermore, as cognitive deficits such as learning impairment and delayed amnesia are debilitating consequences of aging (Forster et al., 1996) it has been reported that chronic administration of antioxidants alleviated age associated cognitive deficits in animals (Allam et al., 2013; Liu et al., 2003; Patki et al., 2015; Solanki et al., 2015).

The aforementioned observations introduce new potential targets for the development of therapeutic interventions based on antioxidant compounds (Scapagnini et al., 2012). In this regard, antioxidant ingredients naturally occurring in the diet and contained in dietary supplements

Abbreviations: ARS, Acute restraint stress; FST, Forced swimming test; SOD, Superoxide dismutase; CAT, Catalase; GPx, Glutathione peroxidase; GR, Glutathione reductase; COX, Cytochrome c oxidase; GSH, Reduced glutathione; GSSG, Oxidized glutathione.

☆ Grant information: Funds for this study were granted by the Spanish Ministry of Education and Science (AGL2008-04892-C03-02).

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http://dx.doi.org/10.1016/j.pnpbp.2015.08.007
0278-5846/

might exert an important role in the aging process as potential drugs to relieve oxidative damage.

The benzopyrones are a group of compounds whose members include coumarins and flavonoids. Dietary exposure to benzopyrones is quite significant as these compounds are found in vegetables, fruits, seeds, nuts, coffee, tea and wine. It is estimated that the average western diet contains approximately 1 g/day of mixed benzopyrones (Lacy and O'Kennedy, 2004). Antioxidant and neuroprotective activities of coumarins have been reported by Jung et al. (2012) based on the suppression of advanced glycation endproducts formation at the level of free radical intermediates and, therefore, on the prevention of its extracellular accumulation in the CNS. The activity of several coumarins on the CNS has been examined showing sedative, anxiolytic, anticonvulsant and antidepressant effects (Peng et al., 2013). For instance, there is convincing evidence that the coumarin scopoletin, administered orally, produces an antidepressant-like effect in the tail suspension test (Capra et al., 2010). Moreover, behavioral and biochemical data from rodents treated with the coumarin nodakenin suggest anti-amnestic properties since it proved ability to ameliorate spatial long term and working memory dysfunction (Kim et al., 2007). Besides, anti-amnestic effects have been observed with the coumarin decursin that are explained by its inhibition of the AChE activity (Kang et al., 2003).

Particularly, esculetin is a coumarin derivative (6,7-dihydroxycoumarin) being present in plants worldwide and dietary supplements (Dulcich and Hartman, 2013; Karmase et al., 2013; Kato et al., 2008; Sung et al., 2014). Previous studies have demonstrated that esculetin possessed antioxidant (Kim et al., 2008; Martín-Aragón et al., 1996) activities and rescued cultured primary neurons from NMDA toxicity (Chang-Ryul et al., 2011; Lee et al., 2011; Wang et al., 2012). We have reported elsewhere a significant increase in the ratio of reduced glutathione/oxidized glutathione (GSH/GSGG) as well as a decrease in content of thiobarbituric acid-reactive substances (TBARs) in liver from C57BL/6J mice upon a chronic esculetin treatment (Martín-Aragón et al., 1998).

In the present work, we hypothesized that a daily consumption of esculetin might have positive effects on mental health and cognitive skills by decreasing oxidative damage to tissues associated with aging, such as the brain cortex, and preventing senescent behavior as a result. Taking into account that emotional reactivity and cognitive function may vary with age (Chen et al., 2006; Doremus et al., 2004), and that the development of successful therapeutics for depression and dementia depends on whether a particular agent retains functional efficacy in aged individuals (Bourin et al., 1998; Homberg et al., 2011; Olivier et al., 2011; Pieramico et al., 2014), the present study was designed to investigate the influence of an esculetin daily treatment in emotional and mood-related behaviors and cognitive performance in mice across different age groups (mature adult and aged mice), in parallel with their brain oxidative status and mitochondrial functionality.

2. Material and methods

2.1. Chemicals

Esculetin (6,7-dihydroxycoumarin) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were of analytical grade and purchased from Sigma-Aldrich and Merck (Germany).

2.2. Animals and treatment schedule

Naïve male C57BL/6J mice (10, 16 and 21 month-old on arrival, Charles River Laboratories International, Inc.) weighing 25–30 g were used. Animas were housed under a 12-h light–dark cycle (lights on at 07:30 h), a temperature of 21 ± 1 °C, a relative humidity of 50–55% and ad libitum access to standard food and tap water except during behavioral tests. Each animal was screened for general health, home cage behavior, sensory abilities, and motor performance (Crawley, 1999). Indices of general health were obtained by measuring body weight and rectal temperature and recording observations of any abnormal physical features (like poorly groomed fur, bald patches in the coat or absence of whiskers). Neurological reflexes that were tested in each mouse included eye blink, ear twitch, whisker twitch, and righting reflex. Animals were allowed to acclimate for at least 14 days before beginning the experiments. The compound to be tested on mice, esculetin, was suspended in a saline solution containing 1% Tween® 80 (Polysorbate 80) and was administered orally through gavage (p.o.) at a volume of 10 ml/kg body weight (25 mg/kg) for 35 consecutive days. Appropriate vehicle-treated groups were assessed simultaneously as controls receiving the same volume of saline solution (containing 1% Tween® 80) and via the same route of administration. The dose of esculetin was selected on the basis of our previous study reports (Martín-Aragón et al., 1998). For instance, doses of 25 and 50 mg/kg esculetin were tested in 11-, 17- and 22-month-old male C57BL/6J mice in a pilot study of behavior paradigms. The dose of 25 mg/kg was chosen for the present study since no statistically significant variations in neurophysiological behavior were found between both dosages.

Mice were divided into six groups with an average of 12 animals per group. The experimental groups were as follows: (1) 11-month-old Control/vehicle + unstressed, 11-month-old Control/vehicle + stressed, 11-month-old Esculetin + unstressed and 11-month-old Esculetin + stressed (2) 17-month-old Control/vehicle + unstressed, 17-month-old Control/vehicle + stressed, 17-month-old Esculetin + unstressed and 17-month-old Esculetin + stressed; (3) 22-month-old Control/vehicle + unstressed, 22-month-old Control/vehicle + stressed, 22-month-old Esculetin + unstressed and 22-month-old Esculetin + stressed. Each group received treatment daily in the morning 10:00 h, for 35 days starting from day 1 after recovery from experimental procedure. At the end of the esculetin treatment, at day 36, mice were sacrificed by decapitation and their brains were quickly removed from the skull for biochemical determinations (as further described). All procedures, including housing, behavioral testing and euthanasia, conformed to the guidelines of The European Directive 2010/63/UE and were approved by the Animal Research Ethical Committee of the Complutense University of Madrid. All efforts were made to minimize the number of animals used and their suffering.

2.3. Acute restraint stress procedure

The acute restraint stress (ARS) is an unavoidable stress situation that may cause anxiogenic behavior (Takeda et al., 1998), depressive-like features (Chourbaji et al., 2008) and disruption of cognitive functions (Guercio et al., 2014) in rodents. Furthermore, these behavioral changes have been associated with an impairment in the antioxidant status in the brain (Kumar and Goyal, 2008). One hour after the esculetin treatment, ARS procedure was performed by a method described previously (Budni et al., 2013; Moretti et al., 2013; Poleszak et al., 2006). Animals were maintained in their home cages with free access to water and food in the period (1 h) that elapsed between the esculetin treatment and ARS procedure. Animals were subjected to an acute stress by immobilization in an individual and well-ventilated rodent restraint device made of Plexiglas fenestrate (10 cm long, 3.2 cm diameter, 0.5 cm wall) (Model CE1032, Cibertec, Spain) for 2 h at a time at room temperature. All physical movements were restrained but mice were not physically squashed and did not undergo pain. The animals were deprived of food and water during the entire period of exposure to stress. Unstressed groups were treated with vehicle or esculetin and were kept without food and water during the entire period of stress. Immediately after restraint, mice were moved back to their home cages. Unstressed mice were placed separately in a different cage for 2 h and then moved back to their home cages. Thirty min after a 2 h-ARS, the animals were released from their enclosure and submitted to the subsequent