Life Sciences 135 (2015) 165–172

Contents lists available at ScienceDirect

Life Sciences

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

Acute restraint stress reduces hippocampal oxidative damage and behavior in rats: Effect of S-allyl cysteine

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ARTICLE INFO

Article history:
Received 8 April 2015
Received in revised form 14 May 2015
Accepted 12 June 2015
Available online 30 June 2015

Keywords:
Oxidative stress
Antioxidant defense
Restraint stress
S-allyl cysteine

ABSTRACT

Aims: This simple study was designed to investigate whether acute restraint stress can generate changes in behavioral tests and hippocampal endpoints of oxidative stress in rats, and if the antioxidant S-allyl cysteine (SAC) can prevent these alterations.
Materials and methods: We evaluated motor activity, forced swimming and anxiety behavior, as well as the hippocampal levels of lipid peroxidation and the activities of glutathione-related enzymes in animals submitted to mild immobilization. The effect of SAC (100 mg/kg, i.p.), given to rats every day 30 min before starting the immobilization session, was also investigated. Immobilization (restraint) stress was induced for a period of 6 h per day for five consecutive days.
Key findings: Our results indicate that, under the tested conditions, acute restraint stimulates compensatory behavioral tasks (motor activity, anxiety and forced swimming) to counteract the stressing conditions prevailing, and selectively increased the levels of lipid peroxidation and the enzyme activities of glutathione-S-transferase (GST) and glutathione peroxidase (GPx) in the hippocampus also as adaptive responses. SAC exhibited preventive effects in the stressed group as it improved behavior, reduced lipid peroxidation and prevented the increase of GST and GPx activities, suggesting that this antioxidant blunted primary pro-oxidative stimuli induced by restraint stress.
Significance: Findings of this work also confirm that the use of antioxidants such as SAC can provide effective protection against the acute oxidative damage associated with anxiety produced by stressing conditions.

1. Introduction

By definition, stress refers as to any given condition affecting the integrity of biological systems. Since acute stress is more related with the expression of adaptive responses, it is characterized by early compensatory responses oriented to restore homeostatic conditions [44] and it provides relevant information on the origin and nature of ongoing harmful events in the nervous system. Stress is often associated with psychiatric (depression, anxiety, and panic) and neurodegenerative disorders (Alzheimer's disease, Parkinson's disease, etc.). Oxidative stress, a condition involving a mishandled excessive formation of reactive oxygen and nitrogen species (ROS/RNS), produces damage of different molecules, organs and tissues, and it has been documented in studies using different models of stress in animals [16,24,25,41,43].

Animal immobilization through different experimental protocols has been employed as a stress-inducing model consisting of deprivation of spontaneous movements [16]. Stress induced by immobilization produces both psychological and physical alterations, triggering oxidative damage in different brain regions, including the hippocampus, brain

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http://dx.doi.org/10.1016/j.lfs.2015.06.009
0024-3205/

cortex and striatum [2,25,28,29,40,49]. Acute immobilization diminishes the activity of antioxidant enzymes – including superoxide dismutase (SOD) – and the levels of reduced glutathione (GSH) [2,41,49]. Consequently, different antioxidants can ameliorate the deleterious events generated by pro-oxidant conditions, therefore supporting their use as therapeutic tools to counteract oxidative damage occurring during stress episodes [10]. We have reported that complete restraint stress induced to rats for 24 h not only resembles the changes in oxidative damage previously reported by others through an increased striatal lipid peroxidation and decreased SOD activity, but also that this stress can be prevented by the antioxidant L-carnitine (L-CAR) and the anti-anxiolytic drug diazepam [32,33]. Therefore, the search for antioxidants with better and wider neuroprotective profiles is pursued.

S-allyl cysteine (SAC) is the most abundant organosulfur molecule found in aged garlic extracts (Fig. 1). SAC exhibits antioxidant properties, including its capacity to scavenge superoxide radical ($O_2^{\bullet-}$) [23,30,31] and to inhibit hydrogen peroxide (H_2O_2) formation [7,21,30]. Through these properties, SAC has been shown to exert neuroprotective effects in different neurotoxic paradigms, including the reduction of the amyloid-beta peptide-induced oxidative damage, learning deficits [39], and apoptosis [37], while it exerted neurotrophic actions on cultured rat hippocampal neurons [36]. SAC also reduced neurotoxicity and oxidative stress during excitotoxic events [38], as well as during depletion of energy metabolism [20]. New clues on the protective actions of SAC have been recently revealed through the neurotoxic model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the rat nervous system. Accordingly, SAC was not only able to reduce oxidative stress [13], but it also exerted modulatory actions on the antioxidant transcription factor Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), thus providing an integral antioxidant response through phase 2 enzymes [14].

The present study is aimed to characterize the effects of SAC on different restraint stress-induced behavioral alterations and endpoints of oxidative activity in the rat hippocampus. We chose the hippocampus because it is known that stress causes the release of corticosteroids in this region, which can modify cognitive aspects of behavioral performance for hours [22].

2. Materials and methods

2.1. Reagents

SAC was synthesized according to previous reports [13,14]. In brief, L-cysteine reacts with allyl bromide and the product was purified by recrystallization from ethanol–water. The final product was compared for its identification with its corresponding standard by melting point. The chemical structure of this compound is shown in Fig. 1. All other reagents were obtained from known commercial sources.

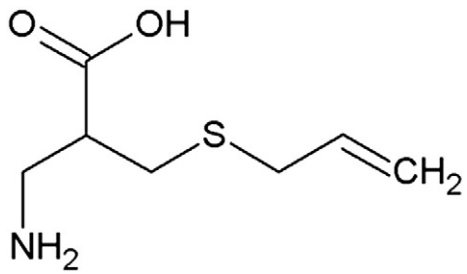

Fig. 1. Schematic representation of the chemical structure of S-allyl cysteine (SAC).

2.2. Animals

All procedures with animals were strictly carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the local guidelines on the ethical use of animals from the Ministry of Health, Mexico. A total of 40 male Wistar rats (260–280 g) were used throughout the study. Animals were obtained from the vivarium of the Faculty of Medicine from the Universidad Nacional Autónoma de México. Rats were kept in polycarbonate cages in the same room where the immobilization protocol was performed. Before the immobilization experiments began, animals were kept in groups of five per cage with free access to food (Laboratory rodent diet 5001; PMI Feeds Inc., Richmond, IN, USA) and water, and under controlled environmental conditions (constant room temperature (25 ± 3 °C), humidity (50 ± 10%) and light/darkness cycles (12:12 h)).

2.3. Restraint stress protocol and drug administration

Animals were randomly assigned to one of four different experimental groups (ten animals per group). In the stress group, immobilization was applied daily for a period of 6 h for 5 consecutive days using an individual rodent restraint device made of plexiglass fenestrate (Model 544-RR, Flat Bottom Restrainer 3.25″ × 8″; Plas Labs Inc., Lansing, MN, USA). This device allowed the full rodent immobilization, during which, animals were completely deprived of food and water. Fig. 2A shows a photograph of the employed device. Control and treatment groups with no stress were isolated, but not subjected to immobilization. For this case, each rat was kept in a small individual cage (30 × 30 × 20 cm) during 6 h for 5 consecutive days. SAC (100 mg/kg, i.p., prepared in distilled water) was administered 30 min before isolation (control condition) or immobilization. The dose of SAC used in this study was the same reported to be effective in an excitotoxic model produced by 3-nitropropionic acid in rats [13]. Every stress session was carried out from 8:00 am to 2:00 pm to avoid any effects due to changes in circadian rhythms. At the end of the immobilization period (5 days), animals were subjected to the behavioral protocols and further sacrificed by decapitation, their brains were collected and their hippocampi were immediately dissected out on ice. Tissue homogenates were obtained and used to estimate different markers of oxidative activity (lipid peroxidation, antioxidant enzymes activity, and protein content).

2.4. Behavioral tests

2.4.1. Motor activity

Motor activity was estimated in a Versamax Animal Activity Monitor and Analyzer open field device (AccuScan Instruments, Inc., Columbus, Ohio; Fig. 2B) for 20 min after the last immobilization event and before removing the animal brains for biochemical analysis, as previously reported [13]. Animals from all groups were deposited in the open plate of the device and observed for 15 min. The collected criteria from the equipment included horizontal and vertical activity, as well as total distance walked. Results were expressed as individual recordings collected along the whole test.

2.4.2. Elevated plus maze

The elevated plus maze was used in this study to evaluate a possible anxiety-related behavior in animals from the different groups, following previous specifications provided for this test [47]. Briefly, 60 min after being submitted to motor activity evaluation and 120 min after challenged in the forced swimming test, rats were deposited in the plus maze device (Fig. 2D) for 5 min and parameters like the time spent and entries made on the open and closed arms were recorded as an index of the preference of the animals for secure and protected areas (closed arms), or for natural instinctive exploration (open arms). The