European Journal of Pharmacology 818 (2018) 300–305

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Molecular and cellular pharmacology

Crocin attenuates acute hypobaric hypoxia-induced cognitive deficits of rats

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ARTICLE INFO

Keywords:
Crocin
Acute hypobaric hypoxia
Cognitive deficits
Hippocampus
PGC-1α
SIRT1

ABSTRACT

This study investigated whether crocin exerted neuroprotective effects against acute hypobaric hypoxia at high altitude in vivo and determined the underlying mechanisms. Male Sprague-Dawley rats were randomly assigned to a normoxic group , a hypoxic group, and three crocin groups at three different doses. The rats were transferred from 50 m to 4200 m for 3 days after treatment with crocin for 3 days. The learning and memory of the rat were evaluated with the Morris water maze test. Transmission electron microscope (TEM) was used to analyze the changes in the ultrastructure of hippocampal neurons. Peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) and sirtuin-1 (SIRT1) levels were determined using immunohistochemical staining and western blotting. The escape latency of the crocin group was shorter than that of the hypoxic group, while the frequency of the rats reaching the platform was significantly higher in the crocin group. The structures of nerve cells and mitochondria were destroyed in the hypoxic group, but were repaired in the crocin groups. The expressions of PGC-1α and SIRT1 were decreased in the hypoxic group, but were increased in the crocin group. All the effects improved by crocin were dose-dependent. Crocin attenuates acute hypobaric hypoxia-induced cognitive deficits in rats, accompanied by repairing the structures of hippocampal neurons and improving PGC-1α and SIRT1 levels.

1. Introduction

The exposure to high altitude is associated with impaired cognitive functions, including memory loss and increased oxidative stress (Jayalakshmi et al., 2007). Acute exposure to high altitudes can cause neurological dysfunction due to decreased oxygen availability to the brain (Shi et al., 2012). Acute hypobaric hypoxia during a short-term exposure to high altitude has been found to cause a decline in cognitive performance, particularly, spatial learning, short-term memory and working memory deficits (Virues-Ortega et al., 2004). Spatial learning and memory in mammals are largely dependent on the hippocampus (Squire, 1992), which is a major area of the brain that is impaired by acute hypobaric hypoxia at high altitude due to a lower partial pressure of oxygen (Roach and Hackett, 2001). Oxidative stress induced by acute hypobaric hypoxia at high altitude caused rapid and severe impairment of hippocampal mitochondrial morphology in rat brain hippocampus (Jain et al., 2015). Recently, an increasing number of studies have indicated that the regulation of mitochondrial biogenesis may be beneficial for impaired cognitive functions (Gray et al., 2016; Palomera-Avalos et al., 2017). However, the important molecules involved in mitochondrial biogenesis against the acute hypobaric hypoxia-induced cognitive deficits remains unclear.

Peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α) has been shown to be a master regulator of mitochondrial biogenesis and energy metabolism (Rodgers et al., 2008). Knockdown of PGC-1α impaired mitochondrial biogenesis and PGC-1α overexpression could rescue mitochondrial deficits in APPswe M17 cells (Sheng et al., 2012). Sirtuin-1 (SIRT1) plays an important role in the deacetylation of PGC-1α, a key transcription coactivator regulating mitochondrial biogenesis (Kim et al., 2014). SIRT1 increases cellular stress resistance, by an increased insulin sensitivity, decreased circulating free fatty acids and increased activity of PGC-1α, and increased mitochondrial number (Corbi et al., 2013). Recently, it has been reported that SIRT1 influences different processes that are potentially involved in the maintenance of brain integrity, such as cell differentiation, DNA repair, cell survival, neurogenesis and neuroprotection (Michan et al., 2010; Ming et al., 2010). Importantly, SIRT1 has been shown to play a critical role in synaptic plasticity and memory formation (Zocchi and Sassone-Corsi, 2012). However, whether PGC-1α and SIRT1 participated in the protection against cognitive deficits induced by acute hypobaric hypoxia needs further investigation.

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http://dx.doi.org/10.1016/j.ejphar.2017.10.042
Received 10 April 2017; Received in revised form 16 October 2017; Accepted 20 October 2017
Available online 26 October 2017
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As the main and active components of *Crocus sativus* L. extract, crocin has been proposed to have effects on learning and memory processes (Khalili and Hamzeh, 2010). Many studies had found that saffron and its active constituent, crocin, can prevent learning and memory impairments as well as the oxidative stress-induced damage to the hippocampus. Others found that crocin improves spatial cognitive abilities (Hosseinzadeh et al., 2012). Thus, these substances may be useful for pharmacological alleviation of cognitive deficits (Ghadrdoost et al., 2011). However, whether crocin can protect against the impaired cognitive functions induced by acute hypobaric hypoxia remained unclear.

To address these questions, we used the rat models exposure to the acute hypobaric hypoxia at high altitudes and intramuscular delivery of crocin in different doses to explore the protective effects of crocin on behavior. We also investigated the changes in the ultrastructure of hippocampal neurons and the expression of SIRT1 and PGC-1α in the rat hippocampus.

2. Materials and methods

2.1. Experimental animals and experimental design

Male pathogen-free Sprague-Dawley (SD) rats were used at 10–12 weeks old and weighed 200–230 g. The rats were obtained from the Laboratory Animal Center of Peking University with certificate number SCXK (Jing 2011-0012). All the rats were randomly assigned to five experimental groups: the normoxic group (n = 6), hypoxic group (n = 6), and crocin (≥ 97% purity, Chengdu Puri Technology Limited, Lot Number: C110113, Chengdu, China) low dose-treated hypoxic group (25 mg/kg/day, n = 6), crocin middle dose-treated hypoxic group (50 mg/kg/day, n = 6), and crocin high dose-treated hypoxic group (100 mg/kg/day, n = 6). Crocin was administered 3 days before the acute hypoxia stimulation by intramuscular drug delivery once per day. The principles governing the care and treatment of animals, as described by the American Physiological Society, were followed at all times during this study and were approved by the Science and Technology Department of Qinghai Province.

The rats in the normoxic group were maintained in a standard environment. The rats in the hypoxic group and treatment groups were exposed to the acute hypoxic environment at 4200 m for 3 days, where the air density was 0.802 kg/m^3, approximately 62% of the air density at sea level. As oxygen varies in direct proportion with air, the oxygen content in the air was also 62% of that at sea level (The Gande County of Qinghai province, China). We observed the acute hypobaric hypoxia-induced cognitive deficits of rats at 4200 m for 3 days after treatment with the drugs. All the rats were given food and water at the same time.

2.2. Morris water maze test

Spatial memory performance was evaluated using the Morris water maze (MWM) test. The rats were subjected to four sessions of training trials daily for five consecutive days (Morris, 1984) before the acute hypoxia stimulation. An overhead camera and a computerized video imaging analysis system (Institute of Materia Medica, Chinese Academy of Medical Sciences, China) were used to record the swimming paths of the rat in the white background of the maze. Daily morning training consisted of 3 trials at intervals of 30 min. The maze consisted of a circular pool (160 cm in diameter, 80 cm deep); the water was maintained at a depth of 50 cm and a temperature of 20–24 °C. The water was made opaque with the addition of powdered milk. A transparent circular platform (8 cm in diameter) was placed 2 cm below the water surface and was fixed in one of the four quadrants throughout training. (Step 1) Place Navigation Test (PNT): On the first day, the rats were trained with free swimming for 2 min to adapt to the pool. Beginning on the second day, a training trial began with the experimenter lowering the animal into the pool with its head facing and close to the side wall, at a position in one of the other three quadrants of the site share quadrant. The timer was started and the time the animal took to escape from the water onto the platform (escape latency) was recorded. If the animal did not find the platform in 60 s, it was picked up by the experimenter and placed onto the platform. The rat remained on the platform for 15 s before being picked up and placed in a holding cage, and the incubation period was recorded as 60 s (Step 2) Spatial Probe Test (SPT): The hidden platform was removed from the pool and the rat was placed in the pool for approximately 120 s. The rats were placed in the pool from the starting point opposite their training quadrant. The frequency was recorded after the animals searched for the platform. The long-term spatial and memory functions were analyzed.

2.3. Transmission electron microscopic study

After observation, the rats were anesthetized with an intraperitoneal injection of 10% chloral hydrate (5 mg/kg), then each rat was decapitated and its brain was immediately removed. After the operation, fresh hippocampal tissues were immersion-fixed in 3% glutaraldehyde for 24 h. The samples were postfixed in 1% osmium tetroxide for 1.5 h, dehydrated through a series of graded ethanol solutions and 1:1 EPON-812 epoxy resin, and then embedded in EPON-812 epoxy resin. Semi-thin sections were stained with toluidine blue, blocks trimmed, and ultrathin sections were stained with lead citrate and uranyl acetate. Specimens were examined using a transmission electron microscope (JEM-1230; Jeol, Japan).

2.4. Immunohistochemical staining

The rat brain was immediately separated and immersed in 4% paraformaldehyde overnight. After washing multiple times, the brain samples were routinely dehydrated and embedded in paraffin. The brain was sectioned at a thickness of approximately 3 μm. The paraffin-embedded sections were de-paraffinized with xylene and rehydrated with ethanol. The sections were incubated with a primary rabbit antibody of anti-rat SIRT1 and PGC-1α (1:100 dilution; Santa Cruz Biotechnology, Inc., USA). The sections were rinsed in PBS three times for 5 min, and sections were incubated with the appropriate biotinylated secondary antibody followed by an avidin–biotin horseradish peroxidase complex (Zhongshan Goldenbridge Ltd., China), with diaminobenzidine (DAB) as substrate. All incubations were performed in a humidified chamber. The negative control sections from each animal received the same staining procedure, except that the primary or secondary antibody was omitted. The tissue sections were photographed and analyzed by the Leica QWinV3 software. The integral optical density (IOD) of the positively stained cells was calculated by the same software. Three random and non-overlapping positively stained microscopic fields were examined in each section of the hippocampal CA1 region using 400× magnification.

2.5. Protein analysis by western blotting

The hippocampus of the rats were dissected and homogenized in ice-cold lysis buffer. The homogenates were subjected to centrifugation at 23646*g* for 15 min at 4 °C, and the protein concentration of the supernatant fluid was quantified using the BCA method (BCA Protein Assay Kit; Thermo Fisher Scientific Inc., USA). The supernatants were incubated at 95 °C in sodium dodecyl sulfate-loading buffer for 5 min before loading onto the gels. Equal amounts of protein (30 μg) were loaded in each lane of 10% SDS-polyacrylamide gels. Following electrophoresis, the proteins were transferred to nitrocellulose membranes and then incubated with 5% skim milk for 2 h. The membranes were incubated with rabbit anti-mouse SIRT1 (1:1500 dilution; Santa Cruz Biotechnology, Inc., USA) and PGC-1α (1:1000 dilution; Santa Cruz Biotechnology, Inc., USA) overnight at 4 °C. After washing, the horseradish peroxidase-conjugated rabbit anti-mouse secondary antibody (1:10000 dilution; Zhongshan Goldenbridge Ltd., China) was applied.

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