Brain Research Bulletin 115 (2015) 1–8

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

Pretreatment with curcumin attenuates anxiety while strengthens memory performance after one short stress experience in male rats

Saida Haider[a,*], Fizza Naqvi[a], Zehra Batool[a,b], Saiqa Tabassum[a,d], Sadia Sadir[a], Laraib Liaquat[a], Faizan Naqvi[a], Nudrat Anwer Zuberi[c], Hina Shakeel[a], Tahira Perveen[a]

[a] Neurochemistry- and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi, Karachi, 75270, Pakistan
[b] Department of Biochemistry, Jinnah University for Women, Karachi-74600, Pakistan
[c] Department of Biochemistry, Jinnah Postgraduate Medical Center (JPMC), Karachi, Pakistan
[d] Department of Biochemistry, Liaquat College of Medicine and Dentistry, Darul-Sehat Hospital, Karachi, Pakistan

ARTICLE INFO

Article history:
Received 22 January 2015
Received in revised form 4 April 2015
Accepted 6 April 2015
Available online 11 April 2015

Keywords:
Curcumin
Acute immobilization stress
Oxidative stress
Memory
Acetylcholinesterase

ABSTRACT

It is observed that memories are more strengthened in a stressful condition. Studies have also demonstrated an association between stressful events and the onset of depression and anxiety. Considering the nootropic, anxiolytic and antidepressant-like properties of curcumin in various experimental approaches, we appraised the beneficial effects of this herb on acute immobilization stress-induced behavioral and neurochemical alterations. Rats in test group were administrated with curcumin (200 mg/kg/day), dissolved in neutral oil, for 1 week. Both control and curcumin-treated rats were divided into unstressed and stressed groups. Rats in the stressed group were subjected to immobilization stress for 2 h. After stress, the animals were subjected to behavioral tests. Immobilization stress induced an anxiogenic behavior in rats subjected to elevated plus maze test (EPM). Locomotor activity was also significantly increased following the acute immobilization stress. Pre-administration of curcumin prevented the stress-induced behavioral deficits. Highest memory performance was observed in stressed rats that were pre-treated with curcumin in Morris water maze (MWM). Brain malondialdehyde (MDA) levels, catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and acetylcholinesterase (AChE) activities were also estimated. Present study suggests a role of antioxidant enzymes in the attenuation of acute stress induced anxiety by curcumin. The findings therefore suggest that supplementation of curcumin may be beneficial in the treatment of acute stress induced anxiety and enhancement of memory function.

1. Introduction

A person can experience a number of emotionally arousing stresses in daily life ranging from acute stress such as exposure to noise, crowd, or public appearance to chronic stress like persistent financial crisis, ongoing work pressure, or loneliness. Acute stress is the reaction to an immediate threat commonly known as fight or flight response. Many studies have reported stress as a predisposing and precipitating factor of deficits (Checkley, 1996; Goyal and Anil, 2007). On the other hand, it is generally accepted that stressful events are very well remembered. Studies with short term exposure to stress have been shown to facilitate learning and memory performance both in animals (Oitzl et al., 2001) and humans (Cahill et al., 2003). This increase in memory performance has been attributed to hormones and neurotransmitters released under stress condition. The activated sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis cause a surge of arousal that is believed to result in memory consolidation following the exposure of stress condition (Joëls et al., 2006). Acute stress that results from traumatic event has been shown to affect several brain activities and makes an individual to feel fear and helplessness, which is equivalent to behavioral despair syndrome in rodents (Poleszak et al., 2006). Acute immobilization stress has been reported to alter locomotor activity and cause anxiety like behaviors (Kumar et al., 2010; Haraguchi et al., 2012). Stress is one of the most important contributory factors in the stimulation of intracellular pathways leading to the increased free radical generation. Numerous reports have revealed that restraint stress can affect central nervous system functions by producing neurochemical and

Abbreviations: AChE, Acetylcholinesterase; ATC, Acetylthiocholine; CAT, Catalase; DTNB, Dithiobisnitrobenzoic acid; EPM, Elevated plus maze; FST, Forced swimming test; GPx, Glutathione peroxidase; MDA, Malondialdehyde; MWM, Morris water maze; NBT, Nitro blue tetrazolium; OFT, Open field test; SOD, Superoxide dismutase; TBA, Thiobarbituric acid; TCA, Trichloroacetic acid.

* Corresponding author at: Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan. Tel.: +92 021 99261316x2577.
E-mail address: saida-h1@hotmail.com (S. Haider).

http://dx.doi.org/10.1016/j.brainresbull.2015.04.001
0361-9230/

hormonal abnormalities associated with imbalance of antioxidant status. The oxidative stress resulting from increased intracellular reactive oxygen species, such as superoxide, hydrogen peroxide, and hydroxyl radicals, disturbs homeostasis within the neurons and can lead to cell death (Uttara et al., 2009). Many studies have shown that restraint stress induces increased lipid peroxidation and increased or decreased antioxidant enzyme activities in different brain regions of rodents depending on the severity and duration of immobilization stress protocol (Fontella et al., 2005; Pajović et al., 2005; Kumari et al., 2007; Atif et al., 2008; Ahmad et al., 2012; García-Fernández et al., 2012). Acute immobilization stress has been reported to induce numerous cellular cascades that lead to increased reactive oxygen species production (Liu et al., 1996). The central nervous system has traditionally been considered as a target site for free radical damage because brain contains abundant lipid content and consumes high amount of oxygen (Halliwell and Gutteridge, 1985).

The psychological deficits associated with acute stressful events may be alleviated using therapeutic strategies involving medicinal and dietary phyto-antioxidants. One such neutraceutical is Curcuma Longa (turmeric) which is widely cultivated in number of Asian countries. Curcumin is the active ingredient and is responsible for yellow color of turmeric (Chattopadhyay et al., 2004; Bala et al., 2006; Aggarwal et al., 2007; Pathak and Khandelwal, 2008). Curcumin is a lipophilic molecule (Hatcher et al., 2008) and can cross the blood brain barrier (Xu et al., 2005). It does not appear to be toxic even at high doses both in humans and animals (Shankar et al., 1980; Soni and Kuttan, 1992; Hatcher et al., 2008). Curcumin has been examined to protect biological membrane from peroxidative damage. The inhibition of lipid peroxidation by curcumin is mainly exhibited due to its scavenging ability of free reactive radical (Ak and Gülçin, 2008). Curcumin is regarded as a potent inducer of detoxifying enzymes and thereby ameliorates oxidative stress (Eybl et al., 2006). Curcumin has diketonic functional groups and polyphenolic structure which determines its lipid peroxidative inhibitor and antioxidant properties (Phan et al., 2001). In several studies it has been used as an antioxidant to reduce the toxicity of various metals including cadmium and lead (Daniel et al., 2004). It has been shown that curcumin ameliorates D-galactose induced cognitive dysfunction (Kumar et al., 2011), inhibits apoptosis, and improves memory function in animal model of Alzheimer's disease (Pan et al., 2008). It has also been reported that curcumin has a role in alleviating stress and depression like symptoms (Kulkarni et al., 2009).

Immobilization stress model merges both emotional and physical aspects of stress so this animal model is widely used to study the brunt of stress on disease process and in stress-associated pathological conditions (Glavin and Hall, 1994). As acute stress and curcumin both have been reported to boost memory function, this study was, therefore, aimed to specifically determine the interactive effects of acute stress and curcumin on memory performance and further to ascertain the ability of curcumin to attenuate the damages of acute immobilization stress and the factors associated with it. Increased arousal level following short term exposure to stressful condition has been shown to improve memory performance. Previously stressful conditions have also shown to induce anxiogenic effects so in the present study we tested our hypothesis that, being a neutraceutical, curcumin may enhance the memory function and reduce anxiogenic effects following acute stress.

2. Material and method

2.1. Animals

Male Albino Wistar rats with mean weight 200 g, bred in Animal House facility of Dow University of Health Sciences (OJHA campus, Karachi, Pakistan), were used for the experiment. Animals were caged individually in plastic cages under standard laboratory conditions and maintained on a 12 h light/dark cycle. Animals had access to cubes of standard rodent diet and tap water *ad libitum* for 3 days prior to acclimatization. The experimental protocols were approved by the institutional ethics and animal care committee and performed in strict accordance with National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Reagents and chemicals

Thiobarbituric acid (TBA), H_2O_2 stock (35%) solution, nitro blue tetrazolium (NBT), trichloroacetic acid (TCA) and dithiobisnitrobenzoic acid (DTNB) were purchased from British Drug House (BDH, Dorset, UK). Acetylthiocholine (ATC), hydroxylamine hydrochloride, curcumin, and all other analytical grade reagents were purchased from Sigma Chemical Co. (St. Louis, USA).

2.3. Treatment schedule

Curcumin was dissolved in neutral oil and administered orally (200 mg/kg) for 7 days whereas the control rats were administered with equal amounts of neutral oil (vehicle) daily. This dose and duration was selected because antioxidant property of curcumin has been documented at this paradigm previously (Emoto et al., 2013). After 1 week both groups (12 rats in each group) were divided into unstressed and stressed rats ($n = 6$). Stressed rats were subjected to immobilization stress for 2 h using restraint tube in separate room whereas the control rats were kept in their home cages. Two hours after stress exposure animals were submitted to behavioral analysis. Forced swimming test (FST), open field test (OFT) and elevated plus maze (EPM) were performed followed by Morris water maze (MWM) test. There was 1 h gap between each behavioral test during which rats were kept in their home cages to avoid overlapping. Control rats were subjected to the same experimental procedures simultaneously. All experiments were carried out between 8:00 and 17:00 h. Rats were decapitated immediately after behavioral tests to collect their brains. Whole brain was removed from the skull within 30 s after decapitation. All brain samples were immediately stored at −70 °C for neurochemical assays. A balanced design was followed for all the treatments and behavioral procedures in order to avoid effect of order and time.

2.4. Immobilization stress protocol

Stress procedure was conducted after 1 week of curcumin administration. Stress groups were subjected to 2 h of immobilization in ventilated, closed plastic tubes that allowed only limited lateral movement (Delaney et al., 2012). Unstressed groups remained in their home cage throughout the duration of the experiment.

2.5. Behavioral tests

2.5.1. Elevated plus maze (EPM) test

Anxiety was assessed by EPM according to the method as described by Naqvi et al. (2012). The apparatus used in the present study was consisted of two closed arms and two open arms with same dimensions (50 × 10 cm). Close arms were enclosed by 40 cm high walls. The arms were connected with a central square (10 × 10 cm) to give the apparatus a plus sign appearance. The maze was elevated 60 cm above the floor. To monitor the activity, rats were individually placed in the central square facing an enclosed arm and the number of entries and time spent in open arm was recorded.