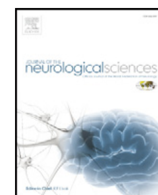
Journal of the Neurological Sciences 302 (2011) 33–42

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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



Antioxidant enzymes are differently changed in experimental ischemic hippocampal CA1 region following repeated restraint stress

Ki-Yeon Yoo [a,1], Choong Hyun Lee [b,1], Joon Ha Park [a], In Koo Hwang [c,*], Ok Kyu Park [d], Seung-Hae Kwon [d], Jung Hoon Choi [e], Dae-joong Kim [f], Young-Guen Kwon [g], Young-Myeong Kim [h], Moo-Ho Won [a,*]

[a] Department of Neurobiology, School of Medicine, Kangwon National University, Chuncheon 200-701, Republic of Korea
[b] Laboratory of Veterinary Pharmacology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea
[c] Department of Anatomy and Cell Biology, College of Veterinary Medicine, and Research Institute for Veterinary Science, Seoul National University, Seoul 151-742, Republic of Korea
[d] Division of Analytical Bio-imaging, Chuncheon Center, Korea Basic Science Institute, Chuncheon, 200-701, Republic of Korea
[e] Department of Anatomy, College of Veterinary Medicine, Kangwon National University, Chuncheon, 200-701, Republic of Korea
[f] Department of Anatomy and Cell Biology, School of Medicine, Kangwon National University, Chuncheon 200-701, Republic of Korea
[g] Department of Biochemistry, College of Sciences, Yonsei University, Seoul 120-749, Republic of Korea
[h] Vascular System Research Center and Department of Molecular and Cellular Biochemistry, School of Medicine, Kangwon National University, Chuncheon 200-701, Republic of Korea

ARTICLE INFO

Article history:
Received 23 July 2010
Received in revised form 2 December 2010
Accepted 7 December 2010
Available online 6 January 2011

Keywords:
Corticosterone
Oxidative stress
Neuronal death
Gliosis
Transient cerebral ischemia
Gerbil

ABSTRACT

Restraint stress induces physiological changes in the brain. In the present study, we observed the effects of repeated stress on ischemic damage associated with oxidative stress in gerbils. Animals were placed into restrainers for 5 h (between 09:30 h and 14:30 h) for 21 consecutive days prior to 5 min of transient cerebral ischemia. Experimental groups were divided into 4 groups; 1) sham-operated control-group (sham-group), 2) ischemia-operated control-group (ischemia-group), 3) sham-operated stress-group (stressed-sham-group), and 4) ischemia-operated stress-group (stressed-ischemia-group). Serum corticosterone level in the ischemia-group was highest (330% vs the sham-group) at 12 h post-ischemia, and serum corticosterone levels in the stressed-ischemia-group were significantly lower than the ischemia-group. Locomotor activity in the ischemia-group was significantly increased (300% vs the sham-group) at 1 day post-ischemia; however, locomotor activity in the stressed-ischemia-group was less increased compared to the ischemia-group. A few NeuN (neuron-specific soluble nuclear antigen)-positive ($^+$) cells were found in the stratum pyramidale (SP) of the hippocampal CA1 region (CA1) 4 days post-ischemia in the ischemia-group; however, in the stressed-ischemia-group at 4 days post-ischemia, 83.8% of NeuN$^+$ neurons were found. In addition, we found a few Fluro-Jade B (a marker for neuronal degeneration)$^+$ and TUNEL$^+$ cells in the stressed-ischemia-group at 4 days post-ischemia. In gliosis, glial fibrillary acidic protein$^+$ astrocytes in the stressed-ischemia-groups was similar to the ischemia-groups; however, ionized calcium-binding adapter molecule 1$^+$ microglia in the stressed-ischemia-groups were much less activated than the ischemia-groups. Among antioxidants, Cu,Zn-superoxide dismutase (SOD1) immunoreactivity in the SP was higher in the stressed-ischemia-groups than the ischemia-groups. Catalase immunoreactivity in the SP of the stressed-ischemia-groups was similar to the ischemia-groups. However, Mn-superoxide dismutase and glutathione peroxidase immunoreactivity were lower than the ischemia-groups. In brief, our results indicate that repeated restraint stress significantly attenuates ischemic neuronal damage and locomotor activity following ischemia. In addition, SOD1 among antioxidants significantly increases in the stressed-ischemia-groups.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Stress can be defined as physical and psychological modifications that disrupt the homeostasis and internal balance of the body. Stress is known to be one of the most important factors in the development of several diseases [1,2]. It has been reported that restraint (immobilization) stress is an easy and convenient method for psychological (escape reaction) and physical stress (muscle work) resulting in restricted mobility and aggression [3,4]. Single or multiple exposures to restraint stress have been regarded as a classic model of stress for over three decades.

* Corresponding authors. Hwang is to be contacted at Department of Anatomy and Cell Biology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, South Korea. Tel.: +82 2 880 1271; fax: +82 2 880 1213. Won, Department of Neurobiology, School of Medicine, Kangwon National University, Chuncheon, 200-701, South Korea. Tel.: +82 33 250 8891; fax: +82 33 256 1614.
E-mail addresses: vetmed2@snu.ac.kr (I.K. Hwang), mhwon@hallym.ac.kr (M.-H. Won).
[1] Contributed equally to this article.

0022-510X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2010.12.006

Restraint stress induces a number of changes in the brain, including the suppression of long-term potentiation, neurogenesis and physiological changes [5–7]. One of the most prominent changes is the release of glucocorticoid hormones from the adrenal cortex, which is controlled by the hypothalamus-pituitary-adrenal system [8]. Glucocorticoids released under stress promote the catabolism of glycogen, muscle, and fat in order to obtain energy required for immediate adaptive response to stress [8].

On the other hand, various stress models have been associated with enhanced free radical generation and altered antioxidant enzyme activities [9–13]. These alterations are explained as a resisting mechanism to the negative effects of reactive oxygen species (ROS), e.g. hydrogen peroxide (H_2O_2), hydroxyl radical (HO) and superoxide anion radical (O^{2-}) that cause the peroxidation of membrane lipids [14,15]. The membrane injury following lipid peroxidation causes disruption of tissue integrity [16]. Therefore, to neutralize ROS, the body uses mainly enzymatic copper,zinc-superoxide dismutase (Cu,Zn-SOD), catalase (CAT) and selenium-dependent glutathione peroxidase (Se-GSH-Px) and non-enzymatic antioxidants, e.g. reduced glutathione (GSH) [17].

There are some reports about influences of different stress models on antioxidant status and lipid peroxidation in rat erythrocytes [11] and effects of cold stress on different tissues [18]. Although stress and its effects on organism have been investigated in several tissues, plasma and erythrocytes in earlier studies [19–21], chronic exposure to stress may be important in various neurological diseases, such as ischemia, Parkinson's disease and Alzheimer's disease. However, there are few reports on effects of chronic restraint stress on ischemic damage together correlations between corticosterone levels, antioxidants and chronic stress.

Stroke has received much interest whether mood disorders, such as depression, and psychological stress can lead to stroke or affect its outcome [22,23]. Several epidemiological studies have reported positive associations between depressive symptoms, psychological stress and stroke risk [6,24–29]. Although a few studies have reported the effects of psychological distress on stroke outcome, these studies have produced controversial results [24,30,31]. Some experimental studies have shown an exacerbation of stroke outcome in animal models of social stress [32,33], while others indicate that stress might protect against ischemic damage [34]. Therefore, we examined the effects of chronic restraint stress on ischemic damage combined with oxidative stress in the hippocampal CA1 region of the Mongolian gerbil after transient cerebral ischemia.

2. Materials and methods

2.1. Experimental animals

Male progeny of Mongolian gerbils (*Meriones unguiculatus*) were obtained from the Experimental Animal Center, Hallym University, Chuncheon, South Korea. Gerbils were used at 6 months (B.W., 65–75 g) of age. The animals were housed in a conventional state under adequate temperature (23 °C) and humidity (60%) control with a 12-h light/12-h dark cycle (lights on at 08:00 h), and provided with free access to food and water. The procedures for handling and caring for animals adhered to the guidelines that are in compliance with the current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996). All of the experiments were conducted to minimize the number of animals used and the suffering caused by the procedures used in the present study.

2.2. Repeated restraint stress

The restraint stress cages were consisted of adjustable length (12.0–2.0 cm length and 3.8 cm diameter) Plexi-glass tubes with air holes in the front, top and back. Gerbils were divided into two groups; control- and repeated restraint stress-groups. Control-group was left untouched in their home cages until the ischemic surgery. Stress-group was placed into restrainers for 5 h (between 09:30 h and 14:30 h) for 21 consecutive days prior to the ischemic surgery. Finally, experimental groups were divided into 4 groups; 1) sham-operated control-group (sham-group), 2) ischemia-operated control-group (ischemia-group), 3) sham-operated stress-group (stressed-sham-group), and 4) ischemia-operated stress-group (stressed-ischemia-group).

2.3. Induction of transient cerebral ischemia

Transient cerebral ischemia was performed at 15:00 h, corresponding to 24 h after the last restraint stress exposure. The animals were anesthetized with a mixture of 2.5% isoflurane in 33% oxygen and 67% nitrous oxide. Bilateral common carotid arteries were isolated and occluded using non-traumatic aneurysm clips. The complete interruption of blood flow was confirmed by observing the central artery in retinae using an ophthalmoscope. After 5 min of occlusion, the aneurysm clips were removed from the common carotid arteries. The body (rectal) temperature under free-regulating or normothermic (37 ± 0.5 °C) conditions was monitored with a rectal temperature probe (TR-100; Fine Science Tools, Foster City, CA) and maintained using a thermometric blanket before, during and after the surgery until the animals completely recovered from anesthesia. Thereafter, animals were kept on the thermal incubator (Mirae Medical Industry, Seoul, South Korea) to maintain the body temperature of animals until the animals were euthanized. Sham-operated animals served as controls: these sham-operated animals were subjected to the same surgical procedures except that the common carotid arteries were not occluded.

2.4. Blood sampling and determination of serum corticosterone levels

To examine serum corticosterone levels in experimental groups after ischemia/reperfusion, blood samples were collected at designated times (12 h, 1 day and 4 days after the surgery) under Equithesin anesthesia via heart in prechilled 1.5 ml Eppendorf tubes. All blood samples were collected between 9:00 and 11:00 a.m. After collection blood samples were centrifuged (5 min, 14,000 rpm, 4 °C) and serum samples were stored in liquid nitrogen until measurement. Serum corticosterone was measured using commercially available RIA kits (IBL, Germany) [49].

2.5. Spontaneous motor activity

To examine the effects of chronic restraint stress against ischemia-induced hyperactivity, spontaneous motor activity in ischemia and stressed-ischemia groups was measured at designated times (1 day, 2 days, 3 days and 4 days after the surgery) according to previous study [35]. For spontaneous motor activity, gerbils ($n = 5$ at each time point) were individually placed in a Plexiglas cage (25 cm × 20 cm × 12 cm), located inside a soundproof chamber. Locomotor activity was recorded with Photobeam Activity System — Home Cage (San Diego Instruments). Gerbils were not exposed to the open field prior to ischemia. Spontaneous motor activity was monitored every 10 min during 60 min and, simultaneously, the traveled distance was quantified by the video signal tracking systems.

2.6. Tissue processing for histology

For the histological analysis, animals were anesthetized with intraperitoneal injection of sodium pentobarbital (30 mg/kg) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The brains were removed and postfixed in the same

Download English Version:

https://daneshyari.com/en/article/1914266

Download Persian Version:

https://daneshyari.com/article/1914266

Daneshyari.com