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Axonal transport proteins and depressive like behavior, following Chronic Unpredictable Mild Stress in male rat

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ABSTRACT

Background: A common mood disorder, depression has long been considered a leading cause of disability worldwide. Chronic stress is involved in the development of various psychiatric diseases including major depressive disorder. Stress can induce depressive-like symptoms and initiate neurodegenerative processes in the brain. The neurodegenerative theory of depression holds impaired axonal transport as a negative factor in neural survival. Axonal transport is a critical mechanism for normal neuronal function, playing crucial roles in axon growth, neurotransmitter secretion, normal mitochondrial function and neural survival.

Methods and materials: To investigate the effects of stress-induced depression, in the present study, we evaluated behavior by forced swimming test (FST), corticosterone plasma level by ELISA assay, hippocampal mRNA expression of three genes (NGF, kinesin and dynein) via real-time PCR and hippocamp count by Nissl staining in male Wistar rats.

Results: Our data demonstrated a significant decrease in the expression of NGF, kinesin and dynein genes in CUMS groups compared to the control group (non-stressed) ($p < 0.05$). CUMS also caused an elevation in immobility time and corticosterone plasma level in the stressed group compared to the controls ($p < 0.01$ and $p < 0.05$, respectively).

Conclusion: The results suggested that the possibility of stress-induced depressive behavior associated with hippocampal neurodegeneration process is correlated with a low expression of kinesin and dynein, the two most important proteins in axonal transport.

1. Introduction

Depression is a prevalent mood disorder nearly all over the world, which can turn into a debilitating condition (1). In 2013, MDD was recognized as the 4th leading cause of disability around the globe, which is suggested to be the second in 2020 [2,3]. The core signs and symptoms of depression include severe sadness, anhedonia, loss of energy, sleep and eating disorders, and impaired personal and social life [4,5]. A rising number of studies has indicated that depression is related to functional and structural changes in specific brain regions [6]. Nerve growth factor (NGF), as a vital neurotropin, is essential for the growth and survival of adult neurons, hippocampal viability and plasticity, and neurite formation. Low levels of NGF result in different conditions, such as depression and depressive like behaviors [7,8]. Moreover, NGF is responsible for proper accumulation of mitochondria during axon genesis in the growing cone [9]. NGF concentration is not the same in different brain areas, but, hippocampus is the structure with one of the highest concentrations [7]. Compared with other somatic cells, neurons are more sensitive to mitochondrial transport impairment [8]. A low concentration of NGF may interrupt normal mitochondrial function [10]. Neurodegeneration theory of depression discusses about neural death [11,12]. Neurotrophic signals, e.g. nerve growth factor (NGF) derived from the specific cells adjacent to the distal-axon areas are carried retrogradely via axonal transport to the cell body. These signals are essential for the maintenance of neurons and cell survival. Disruption of axonal transport precedes the neuronal death and is linked with many neurodegenerative diseases [13,14]. NGF function, especially the distribution pattern is critical for neuron survival [15,16]. Recent

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findings have revealed that circulating and brain NGF levels undergo significant variations after exposure to stressful events [17]. Chronic stress decreases the level of NGF concentration and its distribution pattern concomitantly [18]. Changes in hippocampal expression of NGF mRNA conduce various patterns in different conditions such as neuro-inflammatory and/or neurodegenerative processes. It is believed that even different Chronic Unpredictable Mild Stress (CUMS) protocols contribute differently to changes observed in NGF concentration [19]. In addition, the key role of NGF on axonal transport makes it tricky to find any correlation between NGF expression and the expression of motor proteins. Axonal transport is made possible by the action of 5 different proteins. Kinesin and dynein are the two most important [20,21]. The molecular motors, cytoplasmic dynein and kinesin, which are also ATPases, transport mitochondria toward $-/-$ and $+/-$ ends of microtubules, respectively. On the other hand, one is responsible for anterograde direction along the axon and the other acts in opposite way [22].

Any changes in the expression of these proteins will directly affect mitochondrial transport (axonal transport) and cell function [23]. Normal mitochondrial function is directly and potentially linked to its correct transport [24]. The term “mitochondrial transport” is a complex functional mechanism concerned with mitochondria, which is made up of their movement along the axon (backward and forward), in addition to intracellular buffering and homeostasis control [25,26]. Low expression of kinesin and dynein will impair normal mitochondrial function. Motor proteins act co-dependently [21]. The facts above are in agreement with our suggestion that; possibly down-regulated NGF, kinesin and dynein gene expression are correlated with CUMS-induced depression in rat mold. In neurodegenerative conditions such as Alzheimer, there is a clear finding about impaired mitochondrial movement. Depression and/or stress-induced depressive behavior are types of neurodegenerative condition. It is thought that correction of impaired axonal transport malfunction may modulate this process [27]. In this regard, we investigated NGF and axonal motor protein expression to find any possible effects in depressive-like behavior. This was achieved by applying CUMS protocols on rats to evaluate their behavior, expression of NGF, kinesin, and dynein, and hippocampal cell count by FST, RT-PCR and Nissl staining.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–250 g) were purchased from Razi Institute (Karaj, Iran) and housed under standard laboratory conditions (a 12:12 h light/dark cycle and temperature of 21 ± 1 °C with a humidity of 50 ± 5%.). Animals were allowed to have free access to standard food and water. Twenty rats were equally divided into a stress group and control group. The 10 rats in the stress group received 42 days of CUMS treatment, while the 10 control rats were left undisturbed. All the care and handling of animals were performed according to the guidelines of animal research committee of Iran University of Medical Sciences and were also approved for Ethics in Animal Experiments (No. IR.IUMS.REC. 9221339201).

2.2. Chronic mild stress protocol

The CUMS procedure was conducted as described previously with slight modifications [6,28] (Fig. 1). In brief, rats were exposed to weekly stress regime consisting of: [1] soiled cage, [2] tilting of the cage (45 degree), [3] alterations of the light-dark cycle, tail pinch (1 cm from the end of the tail for 1 min), [4] cold swimming for 5 min (at 4 °C), [5] tail suspension for 5 min, [6] food deprivation for 48 h, [7] water deprivation for 24 h. One of these stressor episodes was applied daily to each rat in a random order for a continuous 42-day period. This pattern of CUMS is expected to result in behavioral abnormalities, equivalent to mental depression in human, which is an acceptable model to study antidepressant medications. The animals in the control group (n = 10) were housed in a separate room with no contact with the stressed rats.

2.3. Behavioral testing

Behavioral tests were performed the day immediately after the 6-week CUMS regime to evaluate depression.

2.4. Forced swim test (FST)

The FST was carried out according to the protocol described in detail in Porsolt et al. with minor modifications [29]. FST set up consisted of a 30 cm deep cylinder (transparent plastic vessel), filled with 16 cm of water (24 ± 1 °C). Rats were unable to touch the bottom of cylinder when floating, although they had the ability to swim. The rats were first placed in water for 15 min (training day), then on the test day, they were immersed in swim tank for five minutes one by one. Each rat was forced to swim for 5 min, and the duration of climbing, immobility, and swimming was recorded using a video camera. The immobility time or immobility behavior (IB) was defined as the duration for which the rat remained floating in a vertical position without struggling and making only slight movements to maintain its head above the water.

2.5. Histological study (Nissl staining)

After the behavioral test, a specific number of animals (both stress and control groups, n = 5) were perfused transcranial to obtain hippocampus samples, which were then fixed in 10% formalin, embedded in paraffin, and sectioned in 7-μm thickness slices by microtome. Samples of hippocampus were stained using Nissl per standard clinical laboratory protocol [30]. Stained sections were observed under optical microscope (Olympus CK2; Olympus Optical Co., Japan). Hippocampal cell count was performed. Cells were counted with Image J Analyzer.

3. Molecular study

3.1. RNA isolation and in vitro transcription

Total RNA from hippocampus tissue (15 mg) was isolated using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. RNA concentration was estimated with measuring optical spectrum in 260 nm and absorbance ratio 260 nm/280 nm confirmed purity. One microgram of total RNA was used as starting material for the complementary deoxyribonucleic acid (cDNA) preparation. RNA was denatured in 70 °C, then immediately cooled. First-strand complementary DNA was synthesized using deoxynucleotide primers and reverse transcriptase according to manufacturer's guide of cDNA kit (Fermantaz, Nederland).

Fig. 1. Schematic overview of the treatment protocol.