Behavioural Brain Research 213 (2010) 218–224

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Resistance to the development of stress-induced behavioral despair in the forced swim test associated with elevated hippocampal Bcl-xl expression

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ARTICLE INFO

Article history:
Received 15 March 2010
Received in revised form 30 April 2010
Accepted 1 May 2010
Available online 8 May 2010

Keywords:
Hippocampus
BDNF
Bax
Bcl-xl
Forced swim test
Immobility

ABSTRACT

Stress may predispose individuals toward depression through down-regulation of neurogenesis and increase in apoptosis in the brain. However, many subjects show high resistance to stress in relation to psychopathology. In the present study, we assessed the possibility that individual-specific patterns of gene expression associated with cell survival and proliferation may be among the molecular factors underlying stress resilience. Brain-derived neurotrophic factor (BDNF), anti-apoptotic B cell lymphoma like X (Bcl-xl) and pro-apoptotic bcl2-associated X protein (Bax) expression were determined in the hippocampus and frontal cortex of rats naturally differed in despair-like behavior in the forced swim test. In the hippocampus, BDNF messenger RNA (mRNA) level was significantly down-regulated 2 h after the forced swim test exposure, and at this time point, Bcl-xl mRNA and protein levels were significantly higher in stressed than in untested animals. The ratios of hippocampal Bcl-xl to Bax mRNA negatively correlated with the total time spent immobile in the test. When animals were divided in two groups according to immobility responses in two consecutive swim sessions and designated as stress resilient if their immobility time did not increase in the second session as it did in stress sensitive rats, it was found that resilient rats had significantly higher Bcl-xl/Bax ratios in the hippocampus than stress sensitive animals. The data suggest that naturally occurring variations in the Bcl-xl/Bax ratio in the hippocampus may contribute to individual differences in vulnerability to stress-induced depression-like behaviors.

1. Introduction

Stressful life events have been suggested as having a substantial relationship with the onset of episodes of major depression [31,40]. Among multiple neurobiological changes triggered by stressor, down-regulation of neurogenesis and increased cell death in central nervous system especially in the hippocampus were implicated in the pathophysiology of stress-related mood disorders [33,37,39,42]. This notion is supported by a variety of animal studies demonstrating decreased proliferation and increased cell death in the hippocampus and cerebral cortex after chronic stress [4,5,13,14,22,23,29,63], which can lead to the development of depression-like state [4,5].

Fortunately, most people are resistant to the development of psychiatric disorders and do not become ill even after serious, long-lasting stressful experiences [33,55]. In line with this, recent rodent studies have shown that 30% of rats exposed to chronic mild stress were resistant to the development of anhedonia, a core symptom of major depression [6]. Mechanisms underlying individual differences in susceptibility to stress-related psychopathology are largely unknown. It is likely that in addition to damaging effects on brain, stressors are capable of inducing changes that counteract the onset of depression [15,19,41]. Some of these changes may be related to cell proliferation and survival. This suggestion is supported by the data linking behavioral effects of chronic antidepressant treatment to enhancement of neurogenesis in the hippocampus [53]. Brain-derived neurotrophic factor (BDNF) and Bcl-2 family members such as highly expressed in the adult brain anti-apoptotic B cell lymphoma like X (Bcl-xl) and pro-apoptotic Bcl-2-associated X (Bax) proteins play an important role in regulation of neuronal cell proliferation and survival [9,30,34,52,54,61]. In most studies, acute and chronic stress decreased BDNF gene expression in the hippocampus [10,20,38,45,47,50,60]. However, in rats resistant to the development of anhedonia under chronic mild stress, BDNF mRNA was up-regulated in the hippocampal CA3 field suggesting involvement of this neurotrophic factor in the mechanism underlying stress resistance of these animals [6]. Much less is known about stress effects on expression of genes encoding apoptotic proteins. Recently, it was shown that a repeated unpredictable stress significantly reduced hippocampal mRNA levels for anti-apoptotic protein Bcl-xl [32].

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doi:10.1016/j.bbr.2010.05.003

We hypothesized that susceptibility to the development of despair-like behaviors under stress may depend on individual-specific patterns of gene expression related to cell survival and proliferation in the brain. In the present study, BDNF, Bax and Bcl-xl gene expression in the hippocampus and frontal cortex, as well as hippocampal Bcl-xl protein expression, plasma adrenocorticotropic hormone (ACTH) and corticosterone levels were determined in rats at 40 min, 2 h and 24 h after the forced swim test. This test consists of the first (pretest) and the second (test) swim sessions [51]. An increase in immobility time in the test session is accepted as an indicator of the behavioral despair [51,59] and thus could be used as a measure of vulnerability to stress.

2. Materials and methods

2.1. Animals

Adult male outbred Wistar rats weighing 254.6 ± 2.4 g were used. The animals were caged singly with free access to food and water. All animal use procedures conformed to international European ethical standards (86/609-EEC) and the Russian national instructions for the care and use of laboratory animals.

2.2. Forced swim test

Depression-like behavior in the forced swim test was induced according to published protocol [51]. Naive rats received a pretest of 15 min followed 24 h later by a 5-min test. Both swim sessions were carried out between 1000 and 1200 h by placing rats in the swim tanks (46 cm × 20 cm glass cylinders) that were filled with water at 25 °C to a depth of 30 cm. Animal behaviors were recorded on videotape and the total duration of immobility during the first 5-min pretest and test sessions was measured later. After swimming, the rats were dried with towels and returned to their home cages.

2.3. Analysis of messenger RNAs for BDNF, Bax and Bcl-xl

Rats were killed by rapid decapitation at 40 min, 2 h and 24 h after the second swim session (test). Control animals were not tested. The hippocampus and frontal cortex were immediately dissected out on a cooled plate and frozen in liquid nitrogen for determination of mRNA levels. Frontal cortex included tissue section 1.5 mm thick cut from the upper surface of the frontal half of the hemispheres. Semi-quantitative RT-PCR described previously [58] was used to determine mRNAs levels. In brief, PCR reactions were conducted using rat-specific primers: for BDNF – forward (5′-cgacgtccctggctggacacttttt-3′) and reverse (5′-agtaagggcccgaacatacgattgg-3′) [8], Bcl-xl – forward (5′-gtgccatcaatggcaacccat-3′) and reverse (5′-ccgccgttctcctggatccaa-3′) [56], Bax – forward (5′-tggttgccctttctactttg-3′) and reverse (5′-gaagtaggaaaggaggccatc-3′) [62], beta-actin – forward (5′-cgtgaaaagatgacccagat-3′) and reverse (5′-attgccgatagtgatgacct-3′) [48]. The PCR products were quantified relatively to beta-actin mRNA by scanning densitometry (Biodoc II Video Documentation System, Biometra GmbH, Gottingen, Germany). The PCR parameters and detection procedures were estimated to provide a linear relationship between the amount of an input template and the amount of PCR product for all tested mRNAs.

2.4. Immunohistochemical analysis of Bcl-xl

Additional two groups of animals, untested and 2 h after the forced swim test ($n = 5$ for each group), were used for this analysis. Rats were deeply anesthetized and perfused transcardially with saline followed by 4% paraformaldehyde. Brains were then removed, cryoprotected by immersion in 30% sucrose and frozen in dry ice-cooled isopentane. Coronal sections through the hippocampus were prepared using a cryostat microtome and mounted on SuperFrost Plus glass slides (Menzel Glaeser, Braunschweig, Germany). Sections were next incubated for 1 day at 4 °C with primary antibody to Bcl-xl (Santa Cruz Biotechnology, USA) at dilution of 1:100. After washing, they were incubated with biotinylated goat anti-rabbit secondary antibody (Santa Cruz Biotechnology, USA) for 16 h at 4 °C and then for 2 h at room temperature with horseradish peroxidase-conjugated streptavidin (Abcam, England) diluted 1:500. Immunoreactivity was visualized using 3,3′-diaminobenzidine as a chromogen. Cells showing a strong positive signal for Bcl-xl were counted in hippocampal CA1, CA3 and dentate gyrus areas recorded by a CCD video camera connected to an Axioskop2 Plus microscope (Zeiss, Germany). Results are expressed as a mean number of Bcl-xl-immunoreactive cells/0.1 mm^2.

2.5. Plasma ACTH and corticosterone measurement

ELISA kits were used for determination of corticosterone (Assay Designs, Inc., USA) and ACTH (Biomerica, Germany) levels in the blood plasma. Trunk blood was collected from rats of the control group and at 40 min, 2 h and 24 h after the forced swim test into cold tubes containing 0.05 ml of 0.25 M EDTA, centrifuged at 4 °C for 20 min, and then the plasma was stored at −60 °C until assay.

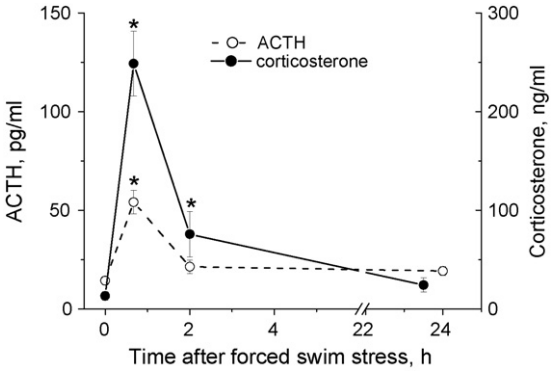

Fig. 1. Effects of acute forced swim stress on plasma ACTH and corticosterone levels. Points represent mean (±SEM) hormone values in untested control rats (0) and at different time intervals after the end of a 5-min stress procedure ($n = 10$/group). *Statistically significant ($p < 0.05$) difference from untested (0) group. ACTH, adrenocorticotropic hormone.

2.6. Statistical analysis

The effects of forced swim stress were analyzed using a one-way ANOVA followed by Fisher's least significant difference (LSD) post hoc test as well as by direct comparisons of groups (Student's t-test). Pearson's correlation analysis was used to evaluate relationships between distinct parameters. The results were considered significant at probability level less than 0.05.

3. Results

3.1. Plasma ACTH and corticosterone levels

Short-term (5 min) exposure to the forced swim test session exerted a marked overall effect on plasma ACTH [$F(3,36) = 23.208$, $p < 0.001$] and corticosterone [$F(3,36) = 28.373$, $p < 0.001$] levels in adult rats (Fig. 1). Post hoc comparisons revealed that both hormones were increased significantly 40 min after the test as compared with those of animals that were not tested. At 2 h, ACTH had returned to basal levels. In contrast to ACTH, a slight increase in corticosterone was still evident 2 h after the forced swim stress exposure.

3.2. BDNF, Bcl-xl and Bax mRNA levels in the hippocampus and frontal cortex

Stress resulting from the forced swim significantly affected BDNF mRNA levels in the hippocampus [$F(3,24) = 11.199$, $p < 0.001$], but not in the frontal cortex [$F(3,26) = 0.632$, ns] (Fig. 2). Post hoc analysis showed a significant reduction of hippocampal BDNF gene expression 2 h after the test. In contrast to the decreasing effect of stress at this time point, BDNF gene expression was up-regulated in the hippocampus 24 h after the test.

Bcl-xl mRNA level was significantly increased in the hippocampus 2 h after the acute forced swim stress [$F(3,27) = 4.426$, $p < 0.05$] (Fig. 3A). No marked differences were found in this gene expression 24 h after the stress procedure in comparison to the controls. In the frontal cortex, Bcl-xl gene expression was not affected by the forced swim [$F(3,26) = 0.972$, ns].

Bax mRNA levels were not significantly altered by the forced swim stress in the hippocampus [$F(3,32) = 0.971$, ns] as well as in the frontal cortex [$F(3,29) = 1.258$, ns] (Fig. 3B).