Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 85 (2006) 842–849
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Chronic mild stress inhibits BDNF protein expression and CREB activation in the dentate gyrus but not in the hippocampus proper

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Received 10 March 2006; received in revised form 24 November 2006; accepted 28 November 2006
Available online 3 January 2007

Abstract

Chronic stress is linked to development of depression and may trigger neurobiological changes underlying the disease. Downregulation of the secretory peptide brain-derived neurotrophic factor (BDNF) and the transcriptional regulator calcium/cyclic-AMP responsive binding protein (CREB) have been implicated in stress and depression-related pathology in animal studies. When animals are exposed to the chronic mild stress (CMS) protocol, multiple depression-like symptoms are observed. Here we investigated the effect of CMS on BDNF protein expression and CREB activation in the dentate gyrus and hippocampus proper. Rats exposed for 5 weeks to repeated, unpredictable, mild stressors showed reduced BDNF expression and inhibited phosphorylation of CREB (Ser-133) in the dentate gyrus ($-25.0\% \pm 3.5\%$ and $-29.7 \pm 7.3\%$, respectively), whereas no significant effects were observed in the hippocampus proper. CMS-treated rats consumed less sucrose compared to control rats, indicating a state of anhedonia. Moreover, phospho-CREB levels in the dentate gyrus were positively correlated with the animals' sucrose intake at the end of the CMS protocol. These results couple chronic mild stress to a downregulation of CREB activity and BDNF protein expression specifically within the dentate gyrus and support the possibility that the BDNF-CREB system plays an important role in the response to environmental challenges.

Keywords: Neurotrophins; Sucrose intake; Anhedonia; CMS; Animal model of depression

1. Introduction

The chronic mild stress (CMS) model has been shown to induce lower consumption of sucrose postulated to reflect anhedonia in animals (diminished capacity to experience pleasure), one of the core symptoms of depression (Willner et al., 1987). The link between taste for sweet solutions and the hedonic state is based on evidence that positive and negative visceral information impact higher level cognitive and behavioural processes (Berntson et al., 2003). 'Taste' is a typical example of brain stem-mediated hedonic evaluation (Badia-Elder et al., 1996; Yamamoto and Sawa, 2000).

The CMS protocol includes mild and uncontrollable daily stressors (e.g. tilted cage, food or water deprivation, paired caging, continuous light, wet bedding). The protocol is regarded as being close to model the human situation, consisting more of daily hassles than traumatic events (see review in (Willner, 2005)). After 4–6 weeks of CMS the animals show a wide variety of symptoms that parallel some features of human

Abbreviations: AMP, adenylmonophosphate; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BSA, bovine serum/albumin; CA, cornus ammonis; CMS, chronic mild stress; CREB, calcium/cyclic-AMP responsive binding protein; EEG, electroencephalogram; EMG, electromyogram; SPD, Sprague-Dawley; TBST, Tris-buffered saline/0.1% Tween-20; TrkB, tyrosine kinaseB; VTA, ventral tegmental area; 5-HT, serotonin; 5-hydroxytryptophan.

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doi:10.1016/j.pbb.2006.11.021

depression, contributing to the face validity of the model (Willner et al., 1987; Willner, 1997). In our experience, exposure of naive rats to CMS induces lower sucrose intake (Gronli et al., 2004, 2005) consistent with anhedonia (Willner et al., 1987), selective changes in sleep (Gronli et al., 2004) consistent with those classically observed in human depression (Benca, 1996), increased locomotor behavior (Gronli et al., 2005) suggested to reflect psychomotor agitation in humans (Ho et al., 2000), and reduced sexual activity (Gronli et al., 2005) also consistent with human depression (Shabsigh et al., 2001). The reliability and the reproducibility of the model have been questioned especially in relation to the inconsistent occurrence of the anhedonic effect as measured by consumption of a sucrose solution (Matthews et al., 1995; Nielsen et al., 2000). Therefore, to confirm the effectiveness of the CMS protocol and in line with our previous reports, the current study also includes the animals' sucrose consumption.

The monoamine hypothesis of depression suggests a deficiency of serotonin (5-hydroxytryptophan, 5-HT) or noradrenaline in the brain (Schildkraut, 1965; Wong and Licinio, 2004). An emerging hypothesis proposes that problems in information processing within specific neural networks in addition to changes in chemical balance may play a critical role in the pathophysiology of depression. Regulation of intracellular messenger cascades mediate the ability of neuronal systems to adapt in response to pharmacological and environmental stimuli and the effect of antidepressants has been suggested to contribute to regaining the plasticity within intracellular signal transduction pathways (Duman et al., 1994). The neurotrophin brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and the most widespread growth factor in the brain. BDNF has diverse functions in the adult brain as a regulator of neuronal survival, fast synaptic transmission, and activity-dependent synaptic plasticity (Lewin and Barde, 1996; Blum and Konnerth, 2005; Bramham and Messaoudi, 2005). A dysregulation of BDNF has been suggested in the pathophysiology of depression (Duman et al., 1997; Altar, 1999; Hashimoto et al., 2004). Levels of serum BDNF are decreased and negatively correlated with the Montgomery-Asberg-Depression Rating Scale in unmedicated major depressive patients (Karege et al., 2002) and are associated with vulnerability to develop mood disorders in healthy subjects (Lang et al., 2004). In rats, downregulation of BDNF mRNA (Smith et al., 1995; Russo-Neustadt et al., 2001; Rasmusson et al., 2002) and BDNF protein (Franklin and Perrot-Sinal, 2006) is found in several brain regions following stress paradigms. Increases in BDNF synthesis and signaling have been implicated in the effect of chronic antidepressant drug treatment (Nibuya et al., 1995; Altar, 1999; Russo-Neustadt et al., 1999; Duman, 2002; Saarelainen et al., 2003; Castren, 2005).

The transcription of the BDNF gene is regulated by the transcriptional regulator calcium/cyclic-AMP responsive-element binding protein (CREB) (Tao et al., 1998; Conti et al., 2002) and BDNF signalling through its receptor tyrosine kinase, TrkB, is capable of inducing CREB phosphorylation (Finkbeiner et al., 1997; Ying et al., 2002). Phosphorylation of CREB at its transcriptional regulatory residue Serine-133 is necessary to activate transcription of genes containing a cAMP response element (Montminy et al., 1990). Like BDNF, activation of CREB is downregulated following stress and upregulated in response to antidepressant treatment (Nibuya et al., 1996; Alfonso et al., 2006).

The hippocampus plays an important role in regulation of stress responses and it expresses high levels of BDNF protein and mRNA in the normal adult rat (Conner et al., 1997). Stress in rats is associated with reduction of hippocampal BDNF levels (Russo-Neustadt et al., 2001; Shirayama et al., 2002). However, there may be a difference between the dentate gyrus and the cornus ammonis (CA) regions of the hippocampus in the stress-induced effect on the BDNF. Immobilization stress in rats is associated with greater impairments in BDNF mRNA expression in the dentate gyrus compared to the CA region (Smith et al., 1995). Furthermore, induced overexpression of CREB in the dentate gyrus, but not in the CA1 or CA3 regions, is associated with antidepressant-like behavioral effects (Chen et al., 2001a,b).

The present study was designed to evaluate a) if CMS affects the hippocampal BDNF-CREB system; b) if a specific difference exists between the dentate gyrus and the CA region; c); if possible changes are correlated to anhedonia-like effects as measured by sucrose intake.

2. Materials and methods

2.1. Ethical evaluation

The experiment has been approved by the Norwegian Animal Research Authority and registered by the Authority. The experiment has thus been conducted in accordance with the laws and regulations controlling experiments in live animals in Norway, i.e. The Animal Protection Act of December 20th 1974, No 73, Chapter VI sections 20–22 and the Animal Protection Ordinance concerning Biological Experiments in Animals of January 15th 1996. Norway has signed and ratified The European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific purposes of March 18, 1986.

2.2. Animal handling

Male Sprague-Dawley (Mol:SPD) rats (Møllegaard, Copenhagen, Denmark) were used in this experiment. To minimize stress, the animals were allowed to remain in the transport cage for five days before they were separated and housed individually in conventional Macrolon type III cages. The home cages were placed in a rack allowing visual, olfactory and auditory contact between animals.

The rats were 11 weeks old prior to the start of the CMS protocol. The rats had free access to food (Rodent low protein diet, B & K Universal AS, Norway) and water, except when the CMS procedure required deprivation. The ambient temperature was 22±1 °C with 52±2% humidity. Rats were kept on a reversed 12 h light/12 h dark schedule with gradually increasing lighting from 1800 h and lights fully on at 1900 h. The rats changed to the reversed L/D schedule 10 days before the start of