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Research Report

Chronic stress induces upregulation of brain-derived neurotrophic factor (BDNF) mRNA and integrin α5 expression in the rat pineal gland

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ABSTRACT

Chronic stress affects brain areas involved in learning and emotional responses. These alterations have been related with the development of cognitive deficits in major depression. Moreover, stress induces deleterious actions on the epithalamic pineal organ, a gland involved in a wide range of physiological functions. The aim of this study was to investigate whether the stress effects on the pineal gland are related with changes in the expression of neurotrophic factors and cell adhesion molecules. Using reverse transcription-polymerase chain reaction (RT-PCR) and Western blot, we analyzed the effect of chronic immobilization stress on the BDNF mRNA and integrin α5 expression in the rat pineal gland. We found that BDNF is produced in situ in the pineal gland. Chronic immobilization stress induced upregulation of BDNF mRNA and integrin α5 expression in the rat pineal gland but did not produce changes in β-actin mRNA or in GAPDH expression. Stressed animals also evidenced an increase in anxiety-like behavior and acute gastric lesions. These results suggest that BDNF and integrin α5 may have a counteracting effect to the deleterious actions of immobilization stress on functionally stimulated pinealocytes. Furthermore, this study proposes that the pineal gland may be a target of glucocorticoid damage during stress.

1. Introduction

Chronic stress induces increased levels of adrenal glucocorticoids and morphologic alterations in limbic areas (McEwen and Chattarji, 2004). The hippocampus, a main structure for spatial learning and memory, is susceptible to stress, and glucocorticoid damage produces dendritic remodeling of CA3 pyramidal neurons and a decrease in adult neurogenesis in the dentate gyrus (Sapolsky et al., 1991; Magariños and McEwen, 1995; McEwen, 1999). More recently, it has been shown that in addition to the hippocampus, the amygdala and prefrontal cortex are morphologically affected by stress and corticosterone in rats (Wellman, 2001; Vyas et al., 2002; Radley et al., 2004). These alterations are related to learning, memory,

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and emotional response impairments (McEwen and Chattarji, 2004). In humans, postmortem and brain imaging studies have revealed atrophy or loss of neurons in the hippocampus and prefrontal cortex of both depressed and anxious patients (Sheline et al., 1996; Shah et al., 1998; McEwen and Chattarji, 2004). Furthermore, hippocampal volume reductions have been related to emotional and memory impairment in major depression (Duman et al., 1999).

Stress has also been found to affect the epithalamic pineal gland, which is known to secrete melatonin, a hormone involved in a wide range of physiological functions (Simonneaux and Ribelayga, 2003). Electron microscopy studies have determined that immobilization stress induces pinealocyte degeneration (Milin et al., 1996; Milin, 1998). These alterations are related with significant increases of rat pineal melatonin levels (Vollrath and Welker, 1998). In tree shrews, psychosocial stress induces a drastic increase of 6-sulfatoxymelatonin (a main melatonin metabolite) excretion in subordinate animals (Fuchs and Schumacher, 1990), while in humans, sleep disturbances, such as insomnia (Jindal and Thase, 2004), and reduced nocturnal peak of pineal melatonin secretion are almost always present in depressed patients (Brown et al., 1985; Frazer et al., 1986; Pacchierotti et al., 2001). These studies suggest that the pineal gland may be target of stress damage by the glucocorticoid stress hormones because this gland expresses high density of the glucocorticoid receptor (Wurembourg, 1975; Sarrieau et al., 1988; Meyer et al., 1998a,b).

Melatonin receptors are present in regions that participate or are affected in the stress response, such as the adrenal glands and the hippocampus, whose activity is modulated by melatonin (Musshoff et al., 2002; Torres-Farfan et al., 2003). Rhythmic melatonin secretion from the pineal has been related to important biological processes such as the modulation of neurotransmitter release, especially serotonin and dopamine (Simonneaux and Ribelayga, 2003). In this line, melatonin has been associated with the regulation of cognitive and emotional processes, such as memory and anxiety (Laudon et al., 1989; Boatright et al., 1994; Hemby et al., 2003).

Stress-related morphologic alterations may be induced by impairments in the neurotrophic factor expression and/or in the neurotrophin signaling transduction pathway. Stress and corticosterone treatment have been found to decrease brain-derived neurotrophic factor (BDNF) mRNA levels in the rat hippocampus and prefrontal cortex (Smith et al., 1995; Nibuya et al., 1999), and BDNF concentration is decreased in the plasma of depressed patients (Karege et al., 2002). BDNF has been shown to protect the central nervous system under a variety of insults and has been considered as a protecting factor in experimental models of depression (Altar, 1999).

Moreover, integrins have been also suggested to play a role in brain damage reparation, especially in response to cellular injury such as focal ischemia (Ellison et al., 1999). Integrins are a family of transmembrane glycoprotein receptors that couple intracellular cytoskeletal elements with extracellular matrix molecules. Integrins exist as $\alpha\beta$ heterodimers that associate at their extracellular domains. Both α and β subunits contribute to the ligand binding domain. The integrin $\alpha 5$ is a key molecular component of the matrix remodeling process after cellular insult (Ellison et al., 1999).

In this context, we decided to study the stress effect on BDNF and integrin $\alpha 5$ expression in the rat pineal gland because both have a key role in the protection to cellular injury in the central nervous system. Thus, the main objective of the present study is to determine whether previously described degenerating pinealocytes and pineal function impairment induced by immobilization stress are associated with changes of BDNF and integrin $\alpha 5$ expression in the rat pineal gland.

2. Results

2.1. *Spontaneous motor responses*

Fig. 1 shows the effects of chronic immobilization stress on spontaneous motor activity. Stress did not affect the motor activity of the rats (control: 1137 ± 67, $n = 12$; stress: 1111 ± 52, $n = 12$) (Fig. 1).

2.2. *Stress markers in the experimental animals*

The behavioral response to chronic stress was investigated comparing the performance of stressed and control rats in the elevated plus-maze test. We observed consistent changes in anxiety levels after exposure to stress. A significant reduction in percentage of open-arm entries (stress: 25.8 ± 3.5%, $n = 12$; control: 40.7 ± 1.7%, $n = 12$; $P < 0.05$) and percentage of time spent in open arms (stress: 15 ± 0.6%, $n = 12$; control: 22 ± 1.4%, $n = 12$; $P < 0.001$) was found in stressed animals. These results are indicative of an enhanced anxiety response in rats exposed to stress compared with control animals (Fig. 2A) and cannot be related to changes in locomotor activity because there was no significant difference in the total number of arm entries. In addition, acute gastric lesions were observed in the stressed animals (Fig. 2B). Stress induced desquamation of the surface epithelium in the stomach of stressed rats that is related with chronic ulcerated lesions. We also analyzed the effects of chronic stress in body and adrenal weights. Statistical analysis revealed a significant reduction in percentage body weight gain ($P < 0.001$; Student's t test)

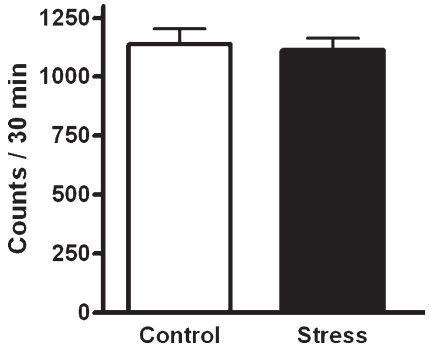

Fig. 1 – Effect of chronic immobilization stress on spontaneous motor responses in rats. Stress does not affect the motor activity of the experimental animals. Bars represent the total spontaneous motor activity in a 30-min observation period. The values are the mean ± SEM.