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Research Report

Differential expression of synaptic proteins after chronic restraint stress in rat prefrontal cortex and hippocampus

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ABSTRACT

Prolonged stress has been associated with altered synaptic plasticity but little is known about the molecular components and mechanisms involved in the stress response. In this study, we examined the effect of chronic restraint stress (CRS) on the expression of genes associated with synaptic vesicle exocytosis in rat prefrontal cortex and hippocampus. Rats were stressed daily using a 21 day restraint stress paradigm, with durations of half an hour or 6 h. RNA and protein were extracted from the same tissue sample and used for real-time quantitative polymerase chain reaction (real-time qPCR) and immunoblotting, respectively. Focusing on the SNARE complex, we investigated the expression of the SNARE core components syntaxin 1A, SNAP-25, and VAMP2 at both transcriptional and protein levels. In addition, the expression of 10 SNARE regulatory proteins was investigated at the transcriptional level. Overall, the prefrontal cortex was more sensitive to CRS compared to the hippocampus. In prefrontal cortex, CRS induced increased mRNA levels of VAMP2, VAMP1, syntaxin 1A, snapin, synaptotagmins I and III, and synapsins I and II, whereas SNAP-25 was down-regulated after CRS. Immunoblotting demonstrated equivalent changes in protein levels of VAMP2, syntaxin 1A, and SNAP-25. In hippocampus, we found increased mRNA levels of VAMP2 and SNAP-29 and a decrease in VAMP1 levels. Immunoblotting revealed decreased VAMP2 protein levels despite increased mRNA levels. Changes in the expression of synaptic proteins may accompany or contribute to the morphological, functional, and behavioral changes observed in experimental models of stress and may have relevance to the pathophysiology of stress-related disorders.

1. Introduction

Stress is a major risk factor for psychiatric disorders. In rodents, chronic stress induces profound behavioral changes manifested as depressive/anxiety-like symptoms and learning and memory deficits, paralleled by structural and functional alterations in specific brain regions and disturbed synaptic plasticity such as changes in the strength or efficacy of synaptic transmission

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Abbreviations: ANOVA, analysis of variance; CRS, chronic restraint stress; ECS, electroconvulsive seizures; NMDA, N-methyl-D-aspartate; RT, room-temperature; Real-time qPCR, real-time quantitative polymerase chain reaction; SNAP, synaptosomal-associated protein; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; VAMP, vesicle-associated membrane protein

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(Pittenger and Duman, 2008). Synaptic transmission relies on the coordinated action between SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins and a number of regulatory synaptic proteins (Jahn and Scheller, 2006; Rizo and Rosenmund, 2008; Südhof, 2004). Exocytosis of synaptic vesicles is promoted by the neuronal SNARE complex, in which the membrane-associated SNAP-25 (synaptosomal-associated protein 25 kDa) and syntaxin 1A interact with VAMP2 (vesicle-associated membrane protein 2) to create a stable ternary core complex (Söllner et al., 1993; Südhof and Rothman, 2009). The SNARE complex provides a bridge between the synaptic vesicle and the plasma membrane, driving the membrane fusion required for exocytosis. The SNARE complex is regulated by a number of synaptic vesicle associated proteins such as the phosphoproteins synapsin I–III, which tether synaptic vesicles to the presynaptic cytoskeletal network, controlling the number of vesicles available for release at the nerve terminus (Fdez and Hilfiker, 2006); the Ca^{2+} binding synaptotagmins I–III, which function as calcium sensors in the regulation of neurotransmitter release (Malsam et al., 2008); SNAP-29, a promiscuous syntaxin-binding SNARE protein, which acts as a negative modulator of neurotransmitter release (Su et al., 2001), and snapin, a SNAP-25 binding protein, which has been suggested to modulate synaptic vesicle exocytosis (Ilardi et al., 1999; Tian et al., 2005).

A number of previous studies have demonstrated changes in gene and protein expression of SNAREs and their regulatory proteins after stress or antidepressant treatment, although with somewhat conflicting results. VAMP2, in particular, has been reported to be targeted by stress and antidepressant treatments but with no apparent consensus in the direction of change in gene or protein expression (Bonanno et al., 2005; Elfving et al., 2008; Gao et al., 2006; Iwamoto et al., 2007; Yamada et al., 2002; Rapp et al., 2004). Synaptophysin, which is generally considered a marker of synaptic density, has been found to be down-regulated in hippocampus after acute and repeated restraint stress (Thome et al., 2001; Xu et al., 2004) and up-regulated after chronic antidepressant treatment (Rapp et al., 2004). However, other studies report no change in hippocampal synaptophysin levels after CRS (Gao et al., 2006) or chronic antidepressant treatment (Bonanno et al., 2005). In severe mental disorders, studies of individual SNARE proteins as well as regulatory proteins indicate abnormalities in prefrontal cortex and hippocampus in schizophrenia (Davidsson et al., 1999; Honer et al., 1999; Harrison, 1999; Fatemi et al., 2001; Thompson et al., 2003), and in hippocampus, frontal cortex, and cingulate cortex, in depression and bipolar disorder (Eastwood and Harrison, 2001; Scarr et al., 2006; Fatemi et al., 2001; Jorgensen and Riederer, 1985; Torrey et al., 2005). These studies suggest that the presynaptic organization and release machinery is targeted by stress and antidepressant treatment and that the changes in levels of synaptic components may represent a form of disturbed synaptic plasticity involved in the pathophysiologies of mood disorders.

To gain further insights into stress-induced alterations in synaptic plasticity at the level of synaptic vesicle exocytosis, we investigated the gene expression profiles of SNARE and SNARE regulatory proteins after CRS in rat prefrontal cortex and hippocampus. Due to a limited amount of protein lysate, immunoblotting was restricted to the analysis of the SNARE proteins syntaxin 1A, SNAP-25, and VAMP2.

2. Results

2.1. *Gene expression in prefrontal cortex*

CRS induced altered transcription levels of all three SNARE complex components: VAMP2, syntaxin 1A, and SNAP-25 (Fig. 1). The VAMP2 mRNA levels were increased to 283%±61% of control levels after 6 h of daily restraint stress. A trend toward increased VAMP2 mRNA expression was also observed in the group subjected to half an hour of daily restraint stress. A similar pattern was observed for syntaxin 1A with transcription levels increasing to 344%±72% of control after 6 h of daily restraint stress and a trend toward increased transcription levels after half an hour of daily restraint stress. Transcription levels of SNAP-25 were reduced to 32%±5% and 42%±6% of control after half an hour and 6 h of CRS, respectively. VAMP1 was the only gene exhibiting an expression profile with a pronounced effect on transcription levels after half an hour (338%±42%) but not after 6 h of daily restraint stress. In addition, CRS increased the transcription levels of 6 of the remaining 9 genes after 6 h of daily restraint stress; SNAP-29 (237%±47%), synaptotagmin I (366%±75%), synaptotagmin III (321%±58%), synapsin I (233%±39%), synapsin II (307%±71%), and snapin (303%±60%). Similar tendencies were observed after half an hour of daily restraint stress but the differences did not reach statistical significance. Synaptophysin was unaffected by CRS, and although there were trends toward increased transcription levels of synaptotagmin II and synapsin III after CRS, they did not reach statistical significance.

2.2. *Gene expression in hippocampus*

In hippocampus, we observed a significant effect on the transcription levels of VAMP1, VAMP2, and SNAP-29 after both half an hour and 6 h of daily restraint stress (Fig. 2). Specifically, VAMP2 mRNA levels were increased to 137%±9% and 136%±5% of control, SNAP-29 mRNA levels were increased to 133%±6% and 128%±7% of control, and VAMP1 mRNA levels were reduced to 83%±2% and 80%±2% of control after half an hour and 6 h of daily restraint stress, respectively. No significant differences were observed in mRNA levels for the two SNARE proteins syntaxin 1A and SNAP-25; the SNARE regulatory proteins snapin and synaptophysin; the phosphoproteins synapsins I, II, and III; or the SNARE associated calcium sensor proteins synaptotagmins I, II, and III.

2.3. *Protein quantification in prefrontal cortex and hippocampus*

In the prefrontal cortex, we found stress-induced changes in protein expression levels equivalent to the changes in mRNA levels observed by real-time qPCR analysis (Fig. 3a–d). Specifically, CRS induced increases in VAMP2 protein levels after half an hour (169%±13%) and 6 h of daily restraint stress