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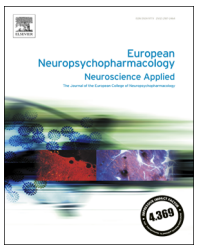

Time-dependent miR-16 serum fluctuations together with reciprocal changes in the expression level of miR-16 in mesocortical circuit contribute to stress resilient phenotype in chronic mild stress – An animal model of depression

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Abstract
MicroRNAs (miRNAs) are involved in stress-related pathologies. However, the molecular mechanisms underlying stress resilience are elusive. Using chronic mild stress (CMS), an animal model of depression, we identified animals exhibiting a resilient phenotype. We investigated serum levels of corticosterone, melatonin and 376 mature miRNAs to find peripheral biomarkers associated with the resilient phenotype. miR-16, selected during screening step, was assayed in different brain regions in order to find potential relationship between brain and peripheral alterations in response to stress. Two CMS experiments that lasted for 2 and 7 consecutive weeks were performed. During both CMS procedures, sucrose consumption levels were significantly decreased in anhedonic-like animals ($p<0.0001$) compared with unstressed animals, whereas the drinking profiles of resilient rats did not change despite the rats being stressed. Serum corticosterone measurements indicated that anhedonic-like animals had blunted hypothalamic-pituitary-adrenal (HPA) axis activity, whereas resilient animals exhibited dynamic responses to stress. miRNA profiling revealed that resilient animals had elevated serum levels of miR-16 after 7 weeks of CMS (adjusted p-value <0.007). Moreover, resilient animals exhibited reciprocal changes in miR-16 expression level in mesocortical pathway after 2 weeks of CMS ($p<0.008$). A bioinformatic analysis showed that miR-16 regulates genes involved in the

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functioning of the nervous system in both humans and rodents. Resilient animals can actively cope with stress on a biochemical level and miR-16 may contribute to a "stress-resistant" behavioral phenotype by pleiotropic modulation of the expression of genes involved in the function of the nervous system.

1. Introduction

Prolonged stress is considered to be one of the major triggers of depression. Nevertheless, many people and laboratory animals exposed to challenging conditions maintain normal behavior and, in the case of humans, cognitive flexibility and optimism (Krishnan et al., 2007; Haglund et al., 2007). These subjects are described as being resilient to stress. Numerous scientific and clinical studies have been performed to explain the pathological changes underlying depression. However, only a few studies have focused on the molecular and neurobiological mechanisms of the stress-resilience phenomenon (McEwen et al., 2015; Feder et al., 2009; Charney, 2004; Issler et al., 2014). Still, little is known about the genetic, epigenetic and biochemical factors responsible for the maintenance of normal behavior and allostatic balance in the face of stress in resilient subjects. Recent advances have revealed that microRNA (miRNA) transcripts play an important role in the physiology of the central nervous system (Jin et al., 2013; Higa et al., 2014) and may be engaged in the pathophysiology of a wide range of neuropsychiatric disorders, including anxiety, depression (Dwivedi, 2014; Mouillet-Richard et al., 2012; Smalheiser et al., 2012) and schizophrenia (Santarelli et al., 2013). Nevertheless, the role of miRNAs in depressed and resilient phenotypes is elusive. miRNAs are short (18–23 nucleotides), non-coding, single-stranded RNA transcripts that are highly evolutionarily conserved among mammals. miRNAs post-transcriptionally regulate gene expression patterns via the down-regulation of their specific targets based on complementarity between the mature miRNA and the 3′ untranslated region (3′UTR) of the targeted mRNA transcript. Impairments of miRNA function in schizophrenic, bipolar (Moreau et al., 2011) and depressed patients (Smalheiser et al., 2012, 2014; Lopez et al., 2014) as well as in stress-vulnerable animals in animal models of depression (Bai et al., 2012; Meerson et al., 2010; Liu et al., 2015a) suggest that miRNAs may play important roles in the regulation of neuronal functioning and in the adaptation of the brain (Dwivedi, 2014). Haramati et al. have shown that acute and chronic stress induce up-regulation of the expression of miR-34c in central amygdala (CA) in mice. This is natural response to a challenging conditions because overexpression of miR-34c in CA caused anxiolytic behavior after challenge (Haramati et al., 2011). A few reports have shown that serum levels of several miRNAs are changed in depressed patients (Li et al., 2013) and that naturalistic stress in healthy persons causes time-dependent changes in miRNA levels in the blood (Honda et al., 2013). Moreover, it has been shown that 12 weeks of antidepressant treatment with escitalopram significantly altered the expression of 30 miRNAs in the blood of depressed patients (Bocchio-Chiavetto et al., 2013). Issler and co-workers have revealed that miR-135a which can negatively regulate the expression of serotonin transporter (SERT) and serotonin receptor 1a (5-HT1a) is involved in antidepressant response in mice. Acute and chronic administration of SSRIs, but not NRIs up-regulated the expression of miR-135a in serotonergic neurons in mice. They also have shown that results from mouse model can be extrapolated into humans since depressed suicides had downregulated the expression levels of miR-135a and miR-16 in the brains. Additionally, patients who suffer from depression had decreased blood level of miR-135a and after 3 months of cognitive behavioral therapy (CBT) they showed significant increase in total blood miR-135a levels as compared to patients receiving only SSRI medication (Issler et al., 2014). Despite the existence of the blood–brain barrier, circulating miRNAs specific for brain tissue can be found extracellularly in biological fluids, such as serum, plasma or CSF (Rong et al., 2011; Liu et al., 2015b), where they exhibit remarkable resistance to degradation (Chen et al., 2008) and may play a potential role as noninvasive biomarkers of mental illness as well as of antidepressant treatment and individual stress responses. Chronic mild stress (CMS), a well-established animal model of depression, can be exploited to investigate the stress-related dysregulation of miRNA functioning, and the results have the appealing potential to be extrapolated to humans and clinical studies because miRNAs are highly conserved among mammals (Mouillet-Richard et al., 2012). CMS uses multiple variable stressors that do not elicit technical habituation and do mimic natural stressors. CMS produces anhedonia, a core symptom of depression that can be measured as decreased sucrose consumption or preference. Moreover, it has been observed that there is a proportion of animals that do not respond to challenging conditions by decreasing their sucrose consumption and instead, maintain their normal behavior (Zurawek et al., 2013). These animals are classified as stress-resilient. Although increasing evidence indicates that peripheral and brain miRNAs are involved in the pathophysiology of depression, little is known about their role in the stress-resilience phenomenon. Moreover, there is still sparse information available on the relationship between central and peripheral changes in miRNA expression resulted in response to stress. Thus, the goal of our work was to investigate time-dependent alterations in a set of 376 mature miRNAs in the serum of rats exposed to a CMS procedure to identify potential, non-invasive, peripheral markers that differentiate the stress-resilient phenotype from the anhedonic-like phenotype. Next, the expression of selected peripheral miRNA markers was examined in ventral tegmental area (VTA), nucleus