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Brain region- and sex-specific modulation of mitochondrial glucocorticoid receptor phosphorylation in fluoxetine treated stressed rats: Effects on energy metabolism

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Summary Antidepressants affect glucocorticoid receptor (GR) functioning partly through modulation of its phosphorylation but their effects on mitochondrial GR have remained undefined. We investigated the ability of chronic fluoxetine treatment to affect chronic stress-induced changes of mitochondrial GR and its phosphoisoforms (pGRs) in the prefrontal cortex and hippocampus of female and male rats. Since mitochondrial GR regulates oxidative phosphorylation, expression of mitochondrial-encoded subunits of cytochrome (cyt) c oxidase and its activity were also investigated. Chronic stress caused accumulation of the GR in mitochondria of female prefrontal cortex, while the changes in the hippocampus were sex-specific at the levels of pGRs. Expression of mitochondrial COXs genes corresponded to chronic stress-modulated mitochondrial GR in both tissues of both genders and to cyt c oxidase activity in females. Moreover, the metabolic parameters in stressed animals were affected by fluoxetine therapy only in the hippocampus. Namely, fluoxetine effects on mitochondrial COXs and cyt c oxidase activity in the hippocampus seem to be conveyed through pGR232 in females, while in males this likely occurs through other mechanisms. In summary, sex-specific regulation of cyt c oxidase by the stress and antidepressant treatment and its differential convergence with mitochondrial GR signaling in the prefrontal cortex and hippocampus could contribute to clarification of sex-dependent vulnerability to stress-related disorders and sex-specific clinical impact of antidepressants.

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1. Introduction

In addition to well-established theories of depression (Gardner and Boles, 2011; Chopra et al., 2011) an additional theory has been proposed during past decade, called ''the mitochondrial dysfunction hypothesis'', suggesting that impaired functions in mitochondria are also associated with psychiatric conditions such as bipolar disorder (Konradi et al., 2004), major depression and a spectrum of other affective disorders (Gardner and Boles, 2011). According to this theory, depression is considered as a disease of mitochondrial energy metabolism (Kato and Kato, 2008) and indeed, an abnormal brain energy metabolism was reported in the frontal and temporal lobes of depressed patients (Deicken et al., 1995) as well as in animal models of depression (Pitman et al., 1988; Alesci et al., 2006).

The glucocorticoid receptor (GR), besides its role in the nucleus, takes part in the coordination of cellular energy requirements and the mitochondrial oxidative phosphorylation enzyme (OXPHOS) biosynthesis (Simoes et al., 2012). In particular, the GR regulates energy metabolism through transcriptional regulation of mitochondrial genes, the cytochrome oxidase 1 (COX 1) and cytochrome oxidase 3 (COX 3), the catalytic subunits of cytochrome (cyt) c oxidase enzyme (Demonacos et al., 1996; Liang et al., 2006). The cyt c oxidase is the terminal respiratory enzyme in the mitochondrial electron transport chain which activity correlates with ATP synthesis and serves as an endogenous metabolic marker for neuronal activity (Wong-Riley, 1989). In line with this, we previously reported that alterations of COX 1 and COX 3 subunits in response to chronic stress were associated with the changes in mitochondrial GR and with its phosphorylation at serine 232 in male rat prefrontal cortex and hippocampus (Adzic et al., 2009).

Alterations of mitochondrial enzymes in mood disorders suggest they could be new biological markers and predictors of response to antidepressants (Hroudova and Fisar, 2010). In particular, cyt c oxidase activity could be considered as an indicator of the antidepressant effects on brain metabolism (O'Reilly et al., 2009). Such findings were supported by in vitro evidence that antidepressant drugs alter the activities of mitochondrial complexes and consequently diminish mitochondrial function (Abdel-Razaq et al., 2011).

Although the latest evidence indicates a sex-specific antidepressant response (Marcus et al., 2005; Sloan and Kornstein, 2003), the clinical applicability of these findings is controversial (Quitkin et al., 2002). It was suggested that women respond better to selective serotonin reuptake inhibitors (SSRI) then to tricyclic antidepressants (Kornstein et al., 2000). Such sex-specific differences in antidepressant response may be related to sexual dimorphisms of the serotonergic system found either in humans (Rubinow et al., 1998) or in animal models of depression (Carlsson and Carlsson, 1988). In agreement with this, we recently demonstrated that sex-specific response to a classical SSRI antidepressant, fluoxetine, could be a consequence of altered nuclear GR signaling by affecting its phosphorylation status (Mitic et al., 2013).

Based on the fact that the brain bioenergetics is necessary therapeutic target in psychiatry for long-term clinical relief and symptom remission and above mentioned reports (Moretti et al., 2003) here we investigate if there is advantage of fluoxetine treatment of females that could be related to the antidepressant-induced alterations of GR signaling in mitochondria and consequently to the cyt c oxidase activity in the prefrontal cortex and the hippocampus, two brain structures significantly affected in stress-related disorders.

2. Methods

2.1. Preparation of fluoxetine-hydrochloride solution

The capsules of Flunirin® were dissolved in sterile distilled water with the aid of ultrasound, and the solution was filtered through Whatman filter paper. The concentration of fluoxetine in the solution was determined using ultra performance liquid chromatography (UPLC) (Djordjevic et al., 2005).

2.2. Animals and treatment

The experiments were performed on adult (three months old) Wistar female and male rats housed four per standard size cage and offered food (commercial rat pellets) and water ad libitum. Light was kept on, between 0700 h and 1900 h and room temperature was kept at 20 ± 2 °C. All animal procedures were approved by the Ethical Committee for the Use of Laboratory Animals of the VINCA Institute of Nuclear Sciences, according to the guidelines of the EU registered Serbian Laboratory Animal Science Association (SLASA). Female and male animals were divided into four experimental groups: two control groups (n = 12, each) and two stressed groups (n = 12, each). The control groups were intraperitoneally treated with the mass-adjusted volume of vehicle (VEH; water)-*control/vehicle group* or with fluoxetine (5 mg/kg per day)-*control/fluoxetine group* between 0900 h and 1000 h for 21 days. In the stressed groups, animals were subjected to the chronic psychosocial isolation for 21 days according to protocol described previously (Adzic et al., 2009; Mitic et al., 2013) and the experiment was performed in two phases. In the first phase, both female and male rats were submitted to the isolation for 21 days and in the second phase, stressed animals were intraperitoneally injected with the mass-adjusted volume of vehicle (VEH; water)-*stress/vehicle group* or with fluoxetine (5 mg/kg per day)-*stress/fluoxetine group* between 0900 h and 1000 h for 21 days, while remained in isolation.

2.3. Determination of the estrous cycle phases

On the day of the experiment, a vaginal smear was taken at approximately 0815 h to establish that the animals were cycling normally (Brack et al., 2006). An additional smear was taken immediately after the animals were sacrificed to determine the cycle stage. Neither of the treatments influenced the distribution of the estrus cycle phases.

2.4. Preparation of brain tissues

All animals were sacrificed between 1000 h and 1100 h, 24 h after the last injection by rapid decapitation. The prefrontal