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PREFRONTAL CORTICAL GLUTATHIONE-DEPENDENT DEFENSE AND PROINFLAMMATORY MEDIATORS IN CHRONICALLY ISOLATED RATS: MODULATION BY FLUOXETINE OR CLOZAPINE

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Abstract—Chronic psychosocial stress modulates brain antioxidant systems and causes neuroinflammation that plays a role in the pathophysiology of depression. Although the antidepressant fluoxetine (FLX) represents the first-line treatment for depression and the atypical antipsychotic clozapine (CLZ) is considered as a second-line treatment for psychotic disorders, the downstream mechanisms of action of these treatments, beyond serotonergic or dopaminergic signaling, remain elusive. We examined behavioral changes, glutathione (GSH)-dependent defense and levels of proinflammatory mediators in the prefrontal cortex (PFC) of adult male Wistar rats exposed to 21 days of chronic social isolation (CSIS). We also tested the ability of FLX (15 mg/kg/day) or CLZ (20 mg/kg/day), applied during CSIS, to prevent stress-induced changes. CSIS caused depressive- and anxiety-like behaviors, compromised GSH-dependent defense, and induced nuclear factor-kappa B (NF-κB) activation with a concomitant increase in cytosolic levels of proinflammatory mediators cyclooxigenase-2, interleukin-1beta and tumor necrosis factor-alpha in the PFC. NF-κB activation and proinflammatory response in the PFC were not found in CSIS rats treated with FLX or CLZ. In contrast, only FLX preserved GSH content in CSIS rats. CLZ not only failed to protect against CSIS-induced GSH depletion, but it diminished its levels when applied to non-stressed rats. In conclusion, prefrontal cortical GSH depletion and the proinflammatory response underlying depressive- and anxiety-like states induced by CSIS were prevented by FLX. The protective effect of CLZ, which was equally effective as FLX on the behavioral level, was limited to proinflammatory components. Hence, different mechanisms underlie the protective effects of these two drugs in CSIS rats.

Key words: chronic social isolation, fluoxetine, clozapine, prefrontal cortex, glutathione, proinflammatory mediators.

INTRODUCTION

An increasing body of evidence suggests that oxidative stress in the brain caused by chronic psychosocial stress contributes to the development of psychiatric disorders, including depression (Van Winkel et al., 2008; Maes et al., 2011; Schiavone et al., 2012). The brain is highly susceptible to oxidative stress due to high oxygen consumption and a lipid-rich environment; thus, maintaining a balanced redox status is crucial for proper brain functioning (Noseworthy and Bray, 1998). In addition, neuroinflammation has been proposed to mediate the association of psychosocial stressors and psychiatric disorders (Maes, 2008; Réus et al., 2015; Calcia et al., 2016). Stress-related activation of inflammatory mediators, as well as modifications of oxidative/nitrosative pathways in the brain, have been implicated in the pathophysiology of psychiatric diseases and may also represent pharmacological targets for their treatment (Munhoz et al., 2008). Chronic social isolation stress (CSIS) is an animal model that has been shown to be reliable for studying the pathophysiology of depression, as it has good face, construct, and predictive validity (Abelaira et al., 2013). It is also widely used as a neurodevelopmental animal model of schizophrenia that produces long-lasting behavioral alterations, such as deficient sensorimotor gating and working memory, locomotor hyperactivity, increased anxiety, and aggression (Fone and Porkess, 2008; Möller et al., 2013). CSIS also produces a variety of neurochemical changes consistent with schizophrenia, including lower frontal cortical dopamine turnover (Heidbreder et al., 2000), altered frontal cortical dopamine D1 and glutamate N-methyl-D-aspartate receptor binding (Toua et al., 2010), and increased striatal dopamine D2 receptor density (King et al., 2009). Together, these behavioral and neurochemical alterations resemble and correspond to features of the human disorder.

The prefrontal cortex (PFC) is one of the most stress-sensitive brain regions (Arnsten, 2009; Sandi, 2013), and

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Abbreviations: ANOVA, analysis of variance; CLZ, clozapine; COX-2, cyclooxygenase-2; CSIS, chronic social isolation; FLX, fluoxetine; GLR, glutathione reductase; GPx, glutathione peroxidase; GSH, glutathione; IL-1β, interleukin-1 beta; iNOS, inducible nitric oxide synthase; IκB, inhibitor kappa B; MB, marble burying; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-kappa B; NOX, nicotinamide adenine dinucleotide phosphate oxidase; PFC, prefrontal cortex; SP, sucrose preference; TNF-α, tumor necrosis factor-alpha; Veh, vehicle.

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a rapidly growing body of evidence implicates this brain structure in the pathophysiology of depression in both clinical and animal models (Ménard et al., 2016; Negrón-Oyarzo et al., 2016). The PFC participates in cognitive, socio-emotional and executive functions and modulates autonomic and neuroendocrine responses to stress (Lucassen et al., 2014). A high density of glucocorticoid receptors (GR), as well as engagement in hypothalamic–pituitary–adrenal (HPA) axis regulation (Smith and Vale, 2006), indicate an important role of the PFC in the response to stress.

Depression is a complex, multifactorial disorder that likely results from the interplay of multiple genetic and environmental factors. Changes in the synthesis, metabolism, reuptake or receptors of neurotransmitters, primarily serotonin, are the focus of a majority of studies concerning the neurobiology of depression (Aan het Rot et al., 2009; Albert et al., 2014). Selective serotonin reuptake inhibitors, which prolong serotonin signaling, are the most widely used treatments for depression, and fluoxetine (FLX) was the first available on the market and is the most commonly used drug of this class (Brambilla et al., 2005; Wagner, 2015). However, 30–40% of patients suffering from major depressive disorder never achieve symptom resolution via standard antidepressant therapy. In some of these cases, atypical antipsychotics used as monotherapy or adjunctively with antidepressants may be effective (Rogóż, 2013; Wang and Si, 2013). Clozapine (CLZ), an atypical antipsychotic with high affinity for serotonin, dopamine, muscarinic, adrenergic and other biogenic amine receptors (Roth et al., 2004), is mainly used in the treatment of psychotic disorders, including schizophrenia, but has been shown to improve symptoms in treatment-resistant bipolar disorder (Li et al., 2015). With regard to FLX, growing evidence demonstrates antioxidative (Behr et al., 2012) as well as immunomodulatory (Baumeister et al., 2015) effects, while CLZ has been reported to induce both pro- and anti-inflammatory activities (Baumeister et al., 2015).

Glutathione (GSH), a non-enzymatic component of GSH-dependent antioxidative defense, plays a central role in maintaining physiological redox status in the brain. It is a substrate for glutathione peroxidase (GPx), which catalyzes the reduction of hydrogen peroxide and a wide variety of organic peroxides to water and the corresponding stable alcohols. Simultaneously, GSH is oxidized to glutathione disulfide, which is reduced back to GSH by glutathione reductase (GLR) (Sastre et al., 2005). Numerous studies have revealed compromised antioxidant defense in the brain of chronically stressed rats (Eren et al., 2007; Ahmad et al., 2010; Che et al., 2015). The results from a proteomic analysis of the PFC revealed that GSH metabolism is among the most altered biological pathways resulting from chronic unpredictable mild stress, a rat model of depression (Yang et al., 2013). Furthermore, Gawryluk et al. (2011) reported reduced GSH levels in post-mortem PFC from patients with bipolar disorder, major depressive disorder and schizophrenia.

In addition to oxidative stress, neuroinflammation plays an important role in the pathophysiology of psychiatric disorders (Smith, 1991; Maes, 2008; Calcia et al., 2016). Patients with depressive disorder exhibit increased expression of proinflammatory cytokines and their receptors in peripheral blood and cerebrospinal fluid (Miller et al., 2009). Moreover, increased expression of interleukin-1 beta (IL-1β) and tumor necrosis factor (TNF) in post-mortem brain samples from depressed patients has been found (Miller and Raison, 2016). IL-1β and TNF-α, major proinflammatory cytokines, are constitutively expressed in healthy, adult brain by neurons and glial cells and act as neuromodulators mediating normal neuronal functions, such as sleep regulation (Vitkovic et al., 2000). Upregulation of these cytokines in the brain is associated with numerous diseases, including mood disorders (McNamara and Lotrich, 2012). A recent study demonstrated that social isolation rearing for 4 weeks elevated rat plasma levels of IL-1β, IL-6 and TNF-α (Ko and Liu, 2015). More importantly, the expression of IL-1β and TNF-α were increased in the frontal cortex of rats that demonstrated anhedonia after 7 weeks of unpredictable mild stress (Liu et al., 2014). These findings are consistent with others regarding the peripheral and central secretion of proinflammatory cytokines in rodent models of depression (Kubera et al., 1998; Leonard and Song, 2002; García-Bueno et al., 2005; Grippo et al., 2005).

The interrelationship between oxidative stress and inflammation in the brain may be mediated by nuclear factor-kappa B (NF-κB), a redox sensitive transcriptional factor that may be activated by both oxidative stress and proinflammatory mediators (Van den Berg et al., 2001; Fischer and Maier, 2015). The activity of NF-κB is under the control of inhibitor kappa B (IκB), which prevents its translocation into the nucleus and subsequent activation of NF-κB target genes (Auphan et al., 1995; Baldwin, 1996; Hayden and Ghosh, 2008). NF-κB is an important positive regulator of inflammatory responses, as it enhances the transcription of proinflammatory cytokines, including TNF-α and IL-1β (Grilli and Memo, 1999; Jin et al., 2008), as well as enzyme cyclooxygenase 2 (COX-2) (Inoue and Tanabe, 1998), which catalyzes the production of prostaglandins, important mediators of inflammation (Marnett et al., 1999; Morgan and Liu, 2011). Interestingly, hydrogen peroxide induces the expression of COX-2, which contributes to further production of reactive oxygen species (Hsieh and Yang, 2013).

Our previous study showed that 21 days of social isolation in adult, male Wistar rats produced features that resemble a depressive-like state, such as anhedonia, anxiety and despair (Zlatković et al., 2014a). Social isolation also compromised antioxidative defense by decreasing the activity of cytosolic copper-zinc superoxide dismutase and mitochondrial manganese superoxide dismutase (Zlatković and Filipović, 2013), and caused oxidative damage of lipids in the PFC (Zlatković et al., 2014a). Here we investigated whether CSIS negatively affects GSH-dependent antioxidative defense by monitoring GSH levels, as well as protein levels and activities of GPx and GLR enzymes. Keeping in mind the close link between oxidative stress and inflammation, we also investigated NF-κB activation and the protein levels of proinflammatory mediators (COX-2, TNF-α and IL-1β) in