Journal of Psychiatric Research 72 (2016) 43–50

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Nod-like receptor pyrin containing 3 (NLRP3) in the post-mortem frontal cortex from patients with bipolar disorder: A potential mediator between mitochondria and immune-activation

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ARTICLE INFO

Article history:
Received 4 June 2015
Received in revised form
8 October 2015
Accepted 22 October 2015

Keywords:
NLRP3
Bipolar disorder
Complex I
Oxidative stress
Post-mortem brain
Frontal cortex

ABSTRACT

Mitochondrial complex I dysfunction, oxidative stress and immune-activation are consistently reported in bipolar disorder (BD). Mitochondrial production of reactive oxygen species was recently linked to activation of an inflammatory redox sensor, the nod-like receptor family pyrin domain-containing 3 (NLRP3). Upon its activation, NLRP3 recruits apoptosis-associated speck-like protein (ASC) and caspase-1 to form the NLRP3-inflamamsome, activating IL-1β. This study aimed to examine if immune-activation may be a downstream target of complex I dysfunction through the NLRP3-inflamamsome in BD. Post-mortem frontal cortex from patients with BD (N = 9), schizophrenia (N = 10), and non-psychiatric controls (N = 9) were donated from the Harvard Brain Tissue Resource Center. Levels of NLRP3, ASC and caspase-1 were measured by western blotting, ELISA and Luminex. While we found no effects of age, sex or post-mortem delay, lower levels of complex I ($F_{2,25} = 3.46$, $p < 0.05$) and NDUFS7, a subunit of complex I ($F_{2,25} = 4.13$, $p < 0.05$), were found in patients with BD. Mitochondrial NLRP3 ($F_{2,25} = 3.86$, $p < 0.05$) and ASC ($F_{2,25} = 4.61$, $p < 0.05$) levels were higher in patients with BD. However, levels of caspase 1 ($F_{2,25} = 4.13$, $p < 0.05$ for both), IL-1β ($F_{2,25} = 7.05$, $p < 0.01$), IL-6 ($F_{2,25} = 5.48$, $p < 0.05$), TNFα ($F_{2,25} = 7.14$, $p < 0.01$) and IL-10 ($F_{2,25} = 5.02$, $p < 0.05$) were increased in both BD and schizophrenia. These findings suggest that immune-activation in the frontal cortex may occur both in patients with BD and schizophrenia, while complex I dysfunction and NLRP3-inflammasome activation may be more specific to BD.

1. Introduction

Oxidative stress occurs when the level of production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) occurs at a greater rate than the ability of antioxidants, such as glutathione, to neutralize them (Cooper and Kristal, 1997; Dringen, 2000; Halliwell, 1992; Sies, 1991). Increased oxidative stress is consistently reported in bipolar disorder (BD) and schizophrenia (SCZ), with studies demonstrating increased protein carbonylation, nitration, cysteine-oxidation, lipid peroxidation, and DNA/RNA oxidation in the post-mortem brain and peripheral samples from patients with BD or SCZ (Andreazza et al., 2007, 2009, 2008; Andreazza et al., 2010; Andreazza et al., 2013; Brown et al., 2014; Clay et al., 2010; Cui et al., 2007; Gawryluk et al., 2011; Kim and Andreazza, 2012; Kim et al., 2014; Moylan et al., 2014; Ng et al., 2008; Savas et al., 2006; Wang et al., 2009). Increased oxidative stress can contribute to damage to mitochondrial proteins, which is particularly vulnerable to oxidation and nitration due to their proximity to sites of ROS and RNS generation, and limited capacity for repair compared to other areas of the cell (Morris and Maes, 2014). For example, ROS damage to mitochondria can produce alterations in the mitochondrial DNA, which could translate to changes in polypeptides (Halliwell and Gutteridge, 2007). In agreement with this, while patients with BD or SCZ do not show symptoms of patients with mitochondrial diseases, studies have reported evidence supporting a potential primary involvement of

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http://dx.doi.org/10.1016/j.jpsychires.2015.10.015
0022-3956/

mitochondrial alterations in the pathophysiology of these disorders, including decreased metabolism in the brain as measured by imaging techniques (Frey et al., 2007; Kato et al., 1993; Maurer et al., 2001), alterations in gene expression of mitochondrial proteins (Iwamoto et al., 2005; Karry et al., 2004; Sun et al., 2006) and mitochondrial DNA polymorphisms (Kenney et al., 2014; Mamdani et al., 2014; Sequeira et al., 2015; Vawter et al., 2006).

On the other hand, patients with BD may also be particularly vulnerable to mitochondrial alterations that result in increased production of ROS from the mitochondria (Kim et al., 2015; Scola et al., 2013). In particular, decreased mRNA and protein levels of subunits that are involved in the transfer of electrons in complex I, which is a member of the electron transport chain, may be a finding that is specific to BD (Iwamoto et al., 2005; Konradi et al., 2012; Scola et al., 2012). In microarray studies, for example, NDUFS7, the final iron-sulfur cluster-containing subunit involved in electron transfer within complex I, was found to be lower in patients with BD (Iwamoto et al., 2005; Scola et al., 2013; Sun et al., 2006). Protein levels of NDUFS7 were also found to be lower in two separate studies examining post-mortem brains from patients with BD (Andreazza et al., 2010, 2013). Decreased efficiency of the electron transfer process within complex I has important implications, as it can result in increased leakage of electrons that react with molecular oxygen to form the superoxide anion (Halliwell and Gutteridge, 2007). The superoxide anion can undergo further reactions to form powerful ROS (Halliwell, 2007). In support of these findings, increased oxidative damage to proteins, lipids and DNA are consistently reported in the brain and periphery of patients with BD (Andreazza et al., 2010, 2013; Clay et al., 2010).

ROS also serve as important signaling molecules through redox sensors. Recent studies have suggested that ROS produced by the mitochondria may be a potent activator of the inflammatory system (López-Armada et al., 2013). Increasing levels of mitochondrial ROS production by inhibiting complex I, for instance, was found to result in greater levels of inflammatory factors such as NF-κB and IL-1β (Li et al., 2003). These findings have important implications for BD, as increased levels of inflammatory cytokines have been found in the brain and periphery of patients with BD (Dean et al., 2013; Kauer-Sant'Anna et al., 2009; Kim et al., 2007; Leboyer et al., 2012; Munkholm et al., 2013; O'Brien et al., 2006; Rao et al., 2012). More specifically, IL-6 and TNF-α were reported to be increased in studies examining peripheral samples from patients with BD (Kim et al., 2007; Munkholm et al., 2013), and studies examining the central nervous system have reported increases in cytokines and receptors involved in the IL-1 pathway (Dean et al., 2013; Rao et al., 2010; Söderlund et al., 2011).

Nod-like receptor pyrin containing 3 (NLRP3) is a redox sensor in the inflammatory system that has been implicated as a potential link between mitochondrial ROS production and immune-activation (Tschopp and Schroder, 2010; Zhou et al., 2011). Indeed, treatment of cells with rotenone, a complex I inhibitor, results in increased production of mitochondrial ROS and subsequently causes the assembly and activation of the NLRP3 inflammasome (Zhou et al., 2011). More specifically, NLRP3, which normally resides in the cytoplasm, undergoes a structural change upon the release of mitochondrial ROS and migrates to the mitochondria, allowing it to be close to the site of damage (Zhou et al., 2011). This structural change allows NLRP3 to recruit two other cytoplasmic proteins - apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1, which form the NLRP3-inflammasome (Schroder et al., 2010; Shimada et al., 2012; Tschopp and Schroder, 2010; Zhou et al., 2011). The assembly of the inflammasome allows for the cleavage of caspase-1 into its active form, releasing it into the cytoplasm to cleave pro-IL-1β and pro-IL-18 to their biologically active forms (Perregaux and Gabel, 1994). IL-1β and IL-18 are then released from the cells to activate downstream pathways involving MYD88 and NF-κB (Eder, 2009), leading to increased expression of other inflammatory molecules such as IL-6 and TNF-α (Bryan et al., 2010; Weber et al., 2010). What remains unknown, however, is whether immune-activation is a downstream target of mitochondrial production of ROS in BD through the NLRP3-inflamamsome. Therefore, in this study, we examined mitochondrial dysfunction, localization and levels of NLRP3, ASC, and caspase-1, and levels of downstream cytokines of the inflammasome pathway in frontal cortex of patients with BD compared to healthy controls.

2. Materials and methods

2.1. Subjects

Frozen post-mortem frontal cortex (BA9,10,24) tissues were generously donated from the Harvard Brain Tissue Resource Centre (Table 1). These well-characterized samples have been in previous publications by our lab and others (Andreazza et al., 2013; Rao et al., 2012). Briefly, subject groups consisted of patients with BD (N = 9), schizophrenia (SCZ; N = 10) and non-psychiatric controls (N = 9). There were no between-group differences for sex ($F_{2,25} = 0.023$, $p = 0.98$), age ($F_{2,25} = 0.18$, $p = 0.83$) and post-mortem interval ($F_{2,25} = 0.14$, $p = 0.87$). Diagnoses were established according to DSM-IV criteria. Samples were coded numerically, and the experimenters were blind to the codes until all the experiments and data analyses were completed.

2.2. Measurement of mitochondrial ETC proteins in the post-mortem frontal cortex

Levels of mitochondrial ETC components complex I (NADH-ubiquinone oxidoreductase), II (succinate ubiquinone oxidoreductase), III (ubiquinone cytochrome c oxidoreductase), IV (cytochrome c oxidase) and V (ATP synthase) and nicotinamide nucleotide transhydrogenase (NNT) were measured using the Human oxidative phosphorylation magnetic bead panel kit (EMD Millipore, #H0XPSMAG-16K) according to manufacturer's instructions. Briefly, tissue extracts were prepared with the lysis buffer provided in the kit followed by centrifugation at 14,000 × g for 20 min. Samples (5ug total protein) were incubated with antibody-coated magnetic beads followed by detection antibodies and streptavidin-phycoerythrin. Luminex Magpix (EMD Millipore) was used to read the plates. Analysis was performed with the xPONENT software, with results expressed as median fluorescence intensity (MFI). NDUFS7 levels were measured by immunoblotting using a previously published protocol (Andreazza et al., 2013). First, brain homogenate was prepared by sonication in a mannitol-sucrose buffer (225 mM mannitol, 75 mM sucrose, 30 mM Tris-HCL at pH7.4) (Wieckowski et al., 2009). 15ug of proteins were loaded onto 12% polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes. Blotting was performed using a primary antibody against NDUFS7 (Santa Cruz, Dallas, TX, USA, sc-98644; 2 h; 1:1000) followed by a secondary goat anti-rabbit IgG horseradish peroxidase antibody (Abcam Inc,

Table 1
Subject information.

	Control (N = 9)	BD (N = 9)	SCZ (N = 10)
Age: mean (range)	79.0 (65–91)	76.1 (58–92)	76.8 (55–91)
PMI: mean (range); hr	21.4 (13.0–30.1)	20.9 (10.0–32.9)	19.4 (8.0–43.5)
Male	4	4	4
Female	5	5	6