Neuroscience Letters 505 (2011) 47–51

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

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

Decreased mRNA expression of uncoupling protein 2, a mitochondrial proton transporter, in post-mortem prefrontal cortex from patients with bipolar disorder and schizophrenia☆

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ARTICLE INFO

Article history:
Received 30 June 2011
Received in revised form
26 September 2011
Accepted 27 September 2011

Keywords:
Bipolar disorder
Schizophrenia
Postmortem
Oxidative stress
Uncoupling protein 2
Superoxide dismutase

ABSTRACT

Although the neurobiological basis of bipolar disorder (BD) remains unknown, mitochondrial dysfunction, oxidative stress and oxidative cell damage have been identified in this disease. Uncoupling proteins (UCP) are proton carriers located in the inner membrane of the mitochondria involved in controlling the production of mitochondrial reactive oxygen species (ROS). Therefore, in this study we wished to investigate the involvement of UCP in BD. We analyzed the RNA and protein levels of UCP2 in the dorsolateral prefrontal cortex (DLPFC) of subjects with BD and schizophrenia (SCZ) and assessed the potential relationship between the antioxidant superoxide dismutase (SOD1 and SOD2) and UCP2 in the same region. Our results showed a downregulation of UCP2 mRNA levels in the DLPFC of subjects with BD and SCZ. There were no differences in UCP2 protein, SOD1 and SOD2 levels between patients and controls. Although more studies are necessary, our results suggest that UCP2 is not been used as a compensatory mechanism to oppose the higher levels of oxidative stress found in BD and SCZ.

Bipolar disorder (BD) is a chronic psychiatric disease characterized by recurrent episodes of hypomania or mania usually alternated with episodes of bipolar depression. Its clinical presentation is complex due to the presence of different subtypes, mixed and psychotic symptoms, rapid cycling and comorbidity [6,31]. Although BD has a neurobiological basis and its causes have been extensively investigated, the detailed pathophysiology of this disease remains unknown [8,28].

To date, numerous studies with diverse approaches have provided evidence for a role of mitochondrial dysfunction in the pathophysiology of BD (for review [14]). Mitochondrial dysfunction may lead to an excessive increase in reactive oxygen species (ROS), resulting in oxidative stress and subsequent oxidative damage to lipids, proteins and nucleic acids [20]. In fact, oxidative damage has been shown postmortem in BD [2,3,10,11,44]. Wang et al. [44] found increased levels of 4-hydroxynonenal (4-HNE), a marker of lipid peroxidation, in anterior cingulate of subjects with BD, while increased oxidation or nitration of mitochondrial proteins has recently been described in frontal cortex in subjects with BD when compared to controls [3]. Furthermore, RNA oxidative damage was significantly increased in hippocampus [11], while DNA fragmentation was increased in non-GABAergic cells of the ACC in subjects with BD [10]. However, the mechanisms associated with mitochondrial dysfunction and oxidative stress in BD are still unclear.

The investigation of these mechanisms has highlighted the possible role of uncoupling proteins (UCP) as regulators of ROS in the cell [5,16,41]. UCPs are proton carriers located in the inner membrane of the mitochondria and they can uncouple oxidation and phosphorylation by facilitating the passage of proton ions from the intermembrane space to the matrix. Since it is well known that an elevated mitochondrial membrane potential increases ROS production, the decrease of this gradient by UCP plays a very important role in reducing the production of ROS by mitochondria [4]. Their

Abbreviations: BD, bipolar disorder; UCP, uncoupling protein; ROS, reactive oxygen species; DLPFC, dorsolateral prefrontal cortex; SCZ, schizophrenia; SOD, superoxide dismutase; 4-HNE, 4-hydroxynonenal.

☆ *Funding sources*: To the execution of this work, Dr. Gigante was supported by a scholarship from the University of Oeste Paulista (UNOESTE), Presidente Prudente, São Paulo, Brazil and by the fellowship program from the Department of Psychiatry of the University of British Columbia, Vancouver, Canada.

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doi:10.1016/j.neulet.2011.09.064

Table 1
Demographic data for postmortem brain tissue.

	CTL	BD	SCZ
Age, Y (mean ± SD)	44.20 ± 7.570	45.29 ± 10.54	42.57 ± 8.465
Range, Y	31–64	19–64	19–59
Gender	26M, 9F	17M, 18F	26M, 9F
PMI, Hr (mean ± SD)	29.37 ± 12.87	37.91 ± 18.34	31.40 ± 15.540
Range (Hr)	9–58	12–84	9–80
pH	6.61 ± 0.27	6.43 ± 0.30	6.47 ± 0.24277
Range	6.0–7.0	5.7–6.9	5.9–6.9

Abbreviation: CTL, control; MDD, major depressive disorder; BD, bipolar disorder; SCZ, schizophrenia; Y, year; M, male; F, female; PM, postmortem hours; Hr, hour.

capacity to control the passage of protons and consequent proton gradient, gives uncoupling proteins an important role in controlling neuromodulation and neuroprotection.

Another question not totally elucidated to date is how UCP2 is activated to increase its neuroprotective action against ROS in the cell environment. Fatty acids and 4-HNE seem to play a role in regulating the levels of UCP2 [12,15,18]. In addition, studies have demonstrated that the superoxide anion has an important role in modulating UCP levels [18] and a positive interaction between superoxide dismutase (SOD) and UCP has been demonstrated [9]. In an important note, SOD is an antioxidant enzyme responsible for reducing superoxide by transforming it into H_2O_2.

Our main purpose in this study was to investigate if UCP might play a role in the control of oxidative balance in BD. Therefore, in this study we measured mRNA and protein levels of UCP2 in the frontal cortex of subjects with BD and SCZ. In addition, we investigated the potential relationship between SOD and UCP2 by measuring protein levels of SOD1 (cytosolic) and SOD2 (mitochondrial) in the same region.

Frozen samples of the dorsolateral prefrontal region (BA9), were obtained from the Stanley Foundation Neuropathology Consortium, MD, USA. Tissue was available from one hemisphere of each brain, with approximately equal numbers sampled in a random manner for each side. Total RNA was also available for each sample. The sample series consisted of 105 subjects (35 controls with no known psychiatric or neurological disorder, 35 SCZ, and 35 BD). Diagnoses were made according to Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria. Detailed demographic, postmortem, and clinical information was available for each case and is reported in Table 1 [43]. All brains underwent clinical neuropathological examination and none demonstrated evidence of neurodegenerative changes or other pathological lesions. One BD case was excluded from this study due to a change in diagnosis.

Frozen tissue was homogenized in 15 volumes of ice-cold tris buffered saline (TBS) and protein quantified using a Lowry-based method (DC assay, BioRad). Total RNA was isolated using Trizol by the Stanley Foundation. RNA integrity and purity were determined with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Received RNA samples were further purified using RNeasy columns (Qiagen, Mississauga, Ont.) and the concentration of RNA for each sample quantified in our laboratory, using the method of Sun et al. [42]. For mRNA expression measurements, eight CTL, three SCZ and eight BD subjects were not included as there was not sufficient RNA sample for respective analyses.

Total RNA isolated from dorsolateral prefrontal cortex was reverse transcribed to cDNA using MuLV reverse transcriptase with random hexamer (Applied Biosystems, Foster City, CA). Gene expression was measured by real-time quantitative amplification RT-PCR using ABI Prism 7300 sequence detection system (Applied Biosystems). The UCP2 gene was amplified with TaqMan MGB probes and gene-specific primers (For ATG ACC ATT AGG TGT TTC GTC TCC; Rev AAG GTG TCC CGT TCT TCA AAG C). mRNA levels were determined using the delta Ct method according to the manufacturer's protocol (Applied Biosystems). Ribosomal 18S RNA was used as an endogenous control for normalization. The PCR reaction for each gene was performed in triplicate.

The levels of uncoupled protein 2, superoxide dismutase 1 and superoxide dismutase 2 were measured by immunoblotting. Briefly, protein extracts were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a 12% acrylamide gel at 100 V for 90 min at 4 °C. Proteins were transferred to polyvinylidene difluoride membranes for 1 h at 100 V and 4 °C. Membranes were dried at room temperature overnight prior to blocking in 5% milk–tris-buffered saline with 0.01% Tween-20 for 1 h at room temperature. Blots were then incubated with primary antibody (UCP2, 1:1000, ab67241; SOD1, 1:1000, ab16831; SOD2, 1:1500, ab13534; Abcam). Following washing, blots were incubated with secondary antibody goat anti-mouse immunoglobulin G (IgG; Abcam) conjugated to horseradish peroxidase (diluted to 1:2000 for UCP2 and SOD1, 1:3000 for SOD2) for 1 h at room temperature. Immunoreactive bands were detected with the enhanced chemiluminescence system using a Fuji LAS-3000 imager. Each gel contained a pre-stained broad range protein ladder (Fermentas International, Ontario, Canada) to measure molecular weights of individual bands. Samples were run blind to diagnosis.

Statistical analyses were computed with PASW Statistics version 18.0 for Windows (Chicago, IL). Normal distribution of data was determined by the Kolmogorov–Smirnov test. Parametric tests were used as data presented a normal distribution. One-way analyses of variance (ANOVA) were employed to analyze data between groups followed by Least Square Derivation post hoc comparisons. The influence of age, post-mortem interval, and pH were determined by analysis of covariance (ANCOVA). Correlations were analyzed by Pearson correlation test. Data are presented as means and standard deviations. Significance was set at $p \leq 0.05$.

Our results showed UCP2 mRNA levels were significantly different between groups ($F_{2,83} = 4.001$, $p = 0.022$). Post hoc analyses indicated decreased UCP2 mRNA levels in patients with SCZ ($p = 0.027$) and BD ($p = 0.010$) when compared to controls (Fig. 1A). UCP2 protein, SOD1 and SOD2 levels were not significantly different between groups ($F_{2,97} = 0.134$, $p = 0.875$; $F = 0.221$, $p = 0.802$: $F = 1.433$, $p = 0.244$) (Fig. 1B–D). Demographic variables are presented in Table 1. We evaluated the correlations between age, PMI and pH with our biochemical parameters (UCP2 mRNA expression or UCP2, SOD1 or SOD2 protein levels) and we did not observe any significant correlation.

To further assess the influence of these potential confounding variables we added age, PMI and pH as covariates in analyses of covariance (ANCOVA). The differences between the groups for UCP2 mRNA levels remained significant ($F = 3.303$, $p = 0.042$), even after these variables were added to the analysis. Post hoc analysis showed that both patients with BD ($p = 0.020$) and SCZ ($p = 0.037$) differed from control subjects. We next examined the effect of pharmacological treatment on UCP2 mRNA and protein levels. Subjects were separated into three groups: (1) prescribed antipsychotics at the time of death, (2) not prescribed antipsychotics at the time of death and (3) control group. The effect of medication was evaluated by ANOVA followed by LSD post hoc analysis. We did not identify any effect of antipsychotic on UCP2 mRNA or protein levels. The same approach was used to evaluate the effect of mood stabilizers or antidepressants on the biochemical parameters and we found no influence of these medications on our results. Furthermore, we found no differences in UCP2 mRNA levels when we separated individuals with psychiatric illness by alcohol/drug abuse. To assess the effect of suicide on UCP2 mRNA we divided the patients into two subgroups: death by suicide ($n = 19$), and death by other causes ($n = 67$). No significant difference between subgroups was observed in UCP2 mRNA levels.