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Mitochondrial respiration in blood platelets of depressive patients

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ABSTRACT

Recent evidences include mitochondrial dysfunctions in pathophysiology of mood disorders. We examined association between depressive disorders and mitochondrial respiration using both intact and permeabilized blood platelets. In intact platelets, physiological respiration, maximal capacity of electron transport system and respiratory rate after complex I inhibition were decreased in depressive patients, who reached partial remission, compared to healthy controls. Respiratory rates were unchanged in several respiratory states in permeabilized platelets. Results indicate that changes in respiratory rate in intact platelets can be used as biological marker of depressive disorder. The hypothesis that decreased mitochondrial respiratory rate participate in pathophysiology of depression was supported.

1. Introduction

Mitochondria provide the most of the energy-rich adenosine 5′triphosphate (ATP) molecules in cells, additionally they are involved in regulation of free radicals, calcium buffering, and redox signaling and take part in intrinsic pathway of apoptosis. A growing body of evidence suggests that mitochondrial dysfunction has an important role in psychiatric disorders, such as major depressive disorder, bipolar disorder, schizophrenia, psychosis, anxiety disorder and borderline personality disorders (Fattal et al., 2006; Gardner and Boles, 2011; Jou et al., 2009; Kato, 2008; Morava et al., 2010; Shao et al., 2008). Psychiatric symptoms often precede the diagnosis of a mitochondrial disease (Fattal et al., 2006, 2007).

The result of impaired mitochondrial functions is complex and includes increased generation of reactive oxygen and nitrogen species, decreased antioxidant levels and changes of balance between antiapoptotic and apoptotic factors (Calabrese et al., 2000; Maes et al., 2012; Starkov, 2008). It is supposed that impaired mitochondrial functions involved in pathophysiology of depressive or bipolar disorder include disturbances in oxidative phosphorylation (OXPHOS), increased mitochondrial DNA (mtDNA) deletions, mutations and/or polymorphisms, impaired calcium signaling, and impaired energy metabolism (Ben-Shachar and Karry, 2008; Frey et al., 2007; Gardner et al., 2003; Kato, 2005, 2008; Stork and Renshaw, 2005). Deletions of mtDNA were found more frequent in patients with depressive disorders compared to controls (Gardner et al., 2003). Nevertheless, the dominant role in the regulation of mitochondrial activity has been put down to nucleus; nuclear-encoded transcription factors control the activity of mitochondrial genome and coordinate expression of nuclear and mitochondrial genes to mitochondrial proteins (Cannino et al., 2007; Reinecke et al., 2009).

The study examining associations between depression and mitochondrial function found that mitochondrial dysfunction is associated with vulnerability to psychopathology in selected depressed patients; antidepressant medication did not seem to influence significantly the mitochondrial biochemical analyses (Gardner et al., 2003). The relationship between mitochondrial dysfunction and unipolar depression has been explored in several studies (Ben-Shachar and Karry, 2008; Gardner and Boles, 2011; Shao et al., 2008). It was demonstrated that muscle mitochondria in depressed patients produced less ATP and activity of complexes I + III and II + III was impaired (Gardner et al., 2003). It was proposed that energy depletion constitutes at least part of the inherited biological predisposition towards the development of depression with somatization (Gardner and Boles, 2008).

Complex I (EC 1.6.5.3) as the main entrance into electron transfer system (ETS) has a major role in control of mitochondrial OXPHOS. Metabolic control analysis based on measurement of oxygen consumption rates and electron transport chain complex activities confirmed that, of the electron transport chain components, complex I as rate-limiting component for oxygen consumption exerts a high level of control over synaptosomal bioenergetics (Telford et al., 2009). In animal model of depression complexes I, III (EC 1.10.2.2) and IV (EC 1.9.3.1) were inhibited in cerebellum and cortex after chronic mild stress, whereas complex II (EC 1.3.5.1) activity was not affected (Rezin et al., 2008). In post mortem study, mRNA and protein levels of complex I subunits were analyzed in human brains of patients suffering from depression and healthy controls. In depressed patients, three subunits of complex I were reduced in cerebellum (Ben-Shachar and Karry, 2008).

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The role of mitochondrial dysfunctions in pathophysiology of depression is supported by the fact that psychotropic medications and/or mitochondrial cocktails are often highly effective at treating both the depressive disorder and mitochondrial diseases (Gardner and Boles, 2011; Parikh et al., 2009). Many psychotropic medications interfere with mitochondrial function (Neustadt and Pieczenik, 2008). In vitro effects of antidepressants on respiratory chain complexes revealed significant decrease of complex I activity (Hroudová and Fišar, 2010, 2011, 2012). However, it remains to be determined if effects of antidepressants and mood stabilizers on mitochondrial functions are related rather to therapeutic or to side effects of the pharmacotherapy.

High-resolution respirometry (Pesta and Gnaiger, 2012) represents a sensitive technique to determine small mitochondrial dysfunctions in depressive disorders. It is mainly performed with a closed-chamber approach, when mitochondrial oxygen consumption causes rapid, easily measurable changes in the concentrations of dissolved oxygen in a closed measuring chamber containing the sample (Horan et al., 2012). From the rate of the oxygen decline the respiratory rate of the mitochondria can be computed. Measurement of respiration during action of appropriate endogenous and exogenous substances enables the identification of the primary sites of effectors and the distribution of control, allowing deeper quantitative analyses (Brand and Nichols, 2011). Respiratory rate near to physiological value can be measured in intact cells in proper medium. The isolated mitochondria or mitochondria in permeabilized cells can be brought into defined "respiratory states" by the sequential addition of substrates, inhibitors and uncouplers. Platelets isolated from peripheral blood can be used as proper model for study of disturbances in cellular respiration (Sjövall et al., 2010).

Impaired function of the OXPHOS may cause disturbances of energy metabolism, which are frequently observed in depressive disorder. There are many possible mechanisms for reduced oxidation rates and ATP production rates that do not include a defect in one of the respiratory chain enzymes (van den Heuvel et al., 2004). Performed study tested the hypothesis that decrease of mitochondrial respiratory rate is linked to depressive disorder. Both intact and permeabilized platelets from peripheral blood were used as a model to study the association of depressive disorder and changes in mitochondrial oxygen consumption measured by high-resolution respirometry.

2. Materials and methods

2.1. Chemicals and solutions

Krebs–Henseleit medium without Ca^{2+} (KH medium) used for dilution of intact platelets in platelet rich plasma consisted of 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4.7H_2O$, 25 mM $NaHCO_3$, and 11.1 mM glucose (pH 7.4); KH medium was saturated with oxygen before use by percolation with 95% O_2 and 5% CO_2 gas mixture.

The mitochondrial respiration medium (MiR05) used in assay with permeabilized platelets consisted of 110 mM sucrose, 60 mM K-lactobionate, 20 mM taurine, 3 mM $MgCl_2.6H_2O$, 10 mM KH_2PO_4, 0.5 mM EGTA, 1 g/l BSA, and 20 mM HEPES, adjusted to pH 7.1 with KOH (Kuznetsov et al., 2004; Pesta and Gnaiger, 2012). Permeabilization of platelet plasma membrane was achieved by addition of digitonin before measurement.

The following stock solutions were used: 10 mg/ml digitonin, 2 M malate, 2 M pyruvate, 0.5 M ADP, 2 M glutamate, 1 M succinate, 4 mg/ml oligomycin, 1 mM and 10 mM carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), 1 mM rotenone, and 0.5 mg/ml antimycin A. Hamilton syringes were used for manual titration of substrates, uncouplers and inhibitors. The chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Study design and participants

Patients with diagnosis of depressive disorder were recruited from the Department of Psychiatry of the First Faculty of Medicine and General University Hospital in Prague. The patients were asked to complete a data set relating to medical history, personal habits and use of medication.

Depressive subjects fulfill the following inclusion criteria: Patients were at age 18 to 65 years, without any organic brain disease, cognitive impairment, and abuse of psychoactive substances. They were without serious somatic disease or chronic somatic pharmacotherapy. Diagnoses of current unipolar depressive episode were confirmed by structured clinical interview for ICD 10; patients were in moderate (F32.1) or severe (F32.2, F32.3) depressive episode, including recurrent depressive disorder, current episode moderate (F33.1) or severe (F33.2, F33.3). Severity of current depression was assessed using the 21-item Hamilton Rating Scale for Depression (HRSD) and Clinical Global Impression-Severity scale (CGI-S); improvement relative to a baseline state at the beginning of the intervention was assessed by Clinical Global Impression-Improvement scale (CGI-I). A negative screen for bipolar disorder was found for all tested subjects using the mood disorder questionnaire (MDQ) (Hirschfeld, 2007).

Patients with depressive disorder were assayed at two different times: firstly at the admission to the inpatient or outpatient treatment, when symptoms of depressive disorder were presented; secondly after significant improvement of clinical picture, when symptoms of depressive disorder largely vanished, i.e. when full or partial remission was reached after several weeks of treatment. The first group was characterized by HRSD score of greater than 10 and CGI-S score 3 or higher; the second group was characterized by HRSD score less than or equal to 10, CGI-S ≤ 3 and CGI-I ≤ 2. A partial remission (or response) was defined as >50% improvement in HRSD score compared to baseline (Hirschfeld et al., 2002). Pharmacotherapy was performed according to the guidelines of the Czech Psychiatric Association (Raboch et al., 2010). Patients were treated with a range of available antidepressants, mostly by selective serotonin re-uptake inhibitors (SSRIs). Some of the patients were treated by mood stabilizers and antipsychotics as augmentation of antidepressive treatment. Control group consisted of age matched healthy volunteers.

The study was carried out according to the principles expressed in the Declaration of Helsinki and the study protocol was approved by the Ethical Review Board of the First Faculty of Medicine and General University Hospital in Prague, Czech Republic. After the nature of the procedures had been fully explained, written informed consent of the participants was obtained.

2.3. Platelets preparation

Peripheral blood samples were taken from the antecubital vein of each fasting participant between 7:00 and 8:00 am, when all subjects were without the use of cigarettes and coffee, and patients were before administration of morning medication. Fifteen milliliters of blood were drawn into BD Vacutainer® blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ 07417, USA) with anticoagulant; buffered sodium citrate was used as an anticoagulant for measuring respiratory rate in intact platelets, and K_3EDTA was used as an anticoagulant for measuring respiratory rate in permeabilized platelets. Platelet rich plasma was separated by centrifugation at 200 ×*g* for 10 min at 25 °C. Platelets were counted by microscopy using a counting chamber and immediately used for measuring mitochondrial respiratory rate.

2.4. High-resolution respirometry

Mitochondrial respiratory rate was measured at 37 °C in a titration-injection high-resolution oxygraph (Oxygraph-2 k, Oroboros Instruments, Innsbruck, Austria) equipped with two closeable tempered

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