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Lithium and valproate modulate energy metabolism in an animal model of mania induced by methamphetamine

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ABSTRACT

Studies have shown alterations in mitochondrial complexes of bipolar disorder (BD) patients. However, changes in the Krebs cycle enzymes have been little studied. The animal model of mania induced by amphetamine has been widely used for the study of bipolar mania. The aim of this study is to assess behavioral and energy metabolism changes in an animal model of mania induced by methamphetamine (m-AMPH). Wistar rats were first given m-AMPH or saline for 14 days, and then, between days 8 and 14, rats were treated with lithium (Li), valproate (VPA), or saline (Sal). Locomotor behavior was assessed using the open-field task and activities of Krebs cycle enzymes (citrate synthase and succinate dehydrogenase), mitochondrial respiratory chain complexes (I, II, III, and IV), and creatine kinase measured in the brain structures (prefrontal, amygdala, hippocampus, and striatum). Li and VPA reversed m-AMPH-induced hyperactivity. The administration of m-AMPH inhibited the activities of Krebs cycle enzymes and complexes of the mitochondrial respiratory chain in all analyzed structures. Li and VPA reversed m-AMPH-induced energetic metabolism dysfunction; however, the effects of Li and VPA were dependent on the brain region analyzed. From the results obtained in this study, we suggested that the decreased Krebs cycle enzymes activity induced by m-AMPH may be inhibiting mitochondrial respiratory chain complexes. Therefore, changes in the Krebs cycle enzymes may also be involved in BD.

1. Introduction

Although bipolar disorder (BD) is a common psychiatric disorder that leads to serious health problems, little is known about its pathophysiology. BD is a multifactorial illness and has diverse symptoms, including recurrences of mania, depression, and mixed states, which hampers the development of a suitable animal model (Machado-Vieira et al., 2004). Despite the difficulties inherent in modeling BD in animals, several behavioral animal models of mania or depression have been developed in an attempt to mimic some aspect of behavioral changes found in this psychiatric condition (Frey et al., 2006a; Jornada et al., 2010; Herman et al., 2007; Machado-Vieira et al., 2004).

Several studies have suggested that dysfunctional cellular energy metabolism has a central role in BD, mainly in the mitochondria (Beech et al., 2010; Maurer et al., 2009; Wang, 2007; Anglin et al., 2012). Abnormalities in energy metabolism were found in functional assays and in magnetic resonance spectroscopy studies (Dager et al., 2004; Deicken et al., 1995; Frey et al., 2007; Regenold et al., 2009). In an animal model of mania, Li and VPA were able to reverse and prevent amphetamine-induced mitochondrial dysfunction, suggesting that one of the mechanisms of action in mood stabilizers may be decreasing the amount of dopamine available, and stabilizing mitochondrial function in the pathophysiology of BD (Valvassori et al., 2010). However, there are few studies evaluating changes in the enzymes of the Krebs cycle in bipolar disorder (Freitas et al., 2010; Fonseca et al., 2005).

Dysfunctions in the Krebs cycle can be capable of altering the rate of brain metabolism and the production of free radicals. After glycolysis, pyruvate is decarboxylated to acetyl CoA by the pyruvate dehydrogenase. The conversion of acetyl CoA to CO_2 in the Krebs cycle

Abbreviations: BD, Bipolar Disorder; AMPHs, Amphetamines; m-AMPH, Metamphetamine; d-AMPH, Dextroamphetamine; Li, Lithium; VPA, Valproate; Sal, Saline; SD, Succinate Dehydrogenase; DCIP, Succinate-2,6-dichloroindophenol; CK, Creatine Kinase; MM-CK, Dimeric MM-creatine kinase; ROS, Reactive Oxygen Species.

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results in the production of NADH for the electron transport chain and subsequent production of ATP. Krebs cycle is a chemical system made up of several enzymes and steps. In the first step, citrate synthase (CS) catalyzes the condensation of oxaloacetate and the acetyl group of acetyl coenzyme-A (Shepherd and Garland, 1996). In the final step of Krebs cycle, malate dehydrogenase (MD) catalyzes the dehydrogenation of l-malate to oxaloacetate (Kelly et al., 1989). Succinate dehydrogenase (SD) is part of both the Krebs cycle and the respiratory chain (complex II); therefore, this enzyme is one of the most important markers of the mitochondrial ability to supply an adequate amount of ATP (Tyler, 1992).

Oxidative phosphorylation is the next step following the Krebs cycle. In the oxidative phosphorylation, electrons are passed along a series of respiratory enzyme complexes (complexes I, II, III, and IV) located in the inner mitochondrial membrane, and the energy released by this electron transfer is used to pump protons across the membrane. The resultant electrochemical gradient enables another complex, adenosine 5′-triphosphate (ATP) synthase, to synthesize ATP from ADP plus Pi (Horn and Barrientos, 2008).

The symptoms of BD involve neurovegetative abnormalities, impulsivity and psychosis, suggesting that anterior limbic brain networks controlling these behaviors are dysfunctional. One of the most important functions of the amygdala is modulate the limbic system, controlling an iterative circuit, prefrontal–striatal–thalamic, which control complex socioemotional behaviors (Strakowski et al., 2000, 2005).

Thus, we examined the activities of mitochondrial enzymes in the Krebs cycle (CS, MD and SD) and respiratory enzyme complexes (complexes I, II, III and IV) in the amygdala, prefrontal, striatum, and hippocampus of rats submitted to an animal model of mania induced by methamphetamine.

2. Experimental methods

2.1. Animals

The subjects were adult male Wistar rats (weighting 250–350 g) obtained from our breeding colony. The animals were housed five to a cage, with food and water available *ad libitum* and were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.) at a temperature of 22 ± 1 °C. All experimental procedures were performed in accordance with, and with the approval of the local Ethics Committee in the use of animals at the Universidade do Extremo Sul Catarinense. All experiments were performed at the same time during the day to avoid circadian variations.

2.2. Drugs and pharmacological procedures

The animals received one daily intraperitoneal injection (i.p.) of m-AMPH 0.25 mg/kg or saline (Sal) for 14 days (45 animals per group). On the 8th day of treatment, the animals in the saline and d-AMPH group were divided in 3 groups (15 animals per group): 1) treatment with Li (47.5 mg/kg i.p.); 2) treatment with VPA (200 mg/kg i.p.) and 3) treatment with Sal for 7 days twice a day for all drugs. On the 15th day of treatment, the animals received a single injection of m-AMPH or Sal and locomotor activity was assessed 2 h after the last injection. The rats were killed by decapitation immediately after the open-field task and amygdala, prefrontal, striatum and hippocampus were dissected, rapidly frozen and stored −70 ºC until assayed.

The dose of Li and VPA was based on previous studies from our laboratory, since Li at 47.5 mg/kg and VPA at 200 mg/kg prevented and reversed the hyperactivity induced by amphetamine. In addition, the animals treated with Li had plasmatic levels of this drug between 0.6 and 1.2 mEq/L, as recommended in the treatment of BD patients (Frey et al., 2006a).

2.3. Locomotor activity

Locomotor activity was assessed using the open-field task as previously described (Barros et al., 2002; Frey et al., 2006a, 2006b, 2006c). This task was performed in a 40×60 cm open field surrounded by 50 cm high walls, made of brown plywood, with the floor divided into 12 equal rectangles by black lines. The animals were gently placed on the left rear rectangle, and left free to explore the arena for 5 min. Crossings of the black lines (locomotor activity/horizontal activity) and rearings (exploratory activity/vertical activity) were counted.

2.4. Tissue and homogenate preparation

The prefrontal cortex, amygdala, hippocampus and striatum were removed and homogenized (1:10, w/v) in SETH buffer, pH 7.4 (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 IU/mL heparin). The homogenates were centrifuged at $800 \times g$ for 10 min at 4 °C and the supernatants kept at −70 °C until being used for enzyme activity determination. The maximal period between homogenate preparation and enzyme analysis was always less than 5 days. The protein content was determined by the method described by Lowry et al. (1951) using bovine serum albumin as standard.

2.5. Activities of enzymes of Krebs cycle

2.5.1. Citrate synthase activity

Citrate synthase activity was assayed according to the method described by Shepherd and Garland (1996). The reaction mixture contained 100 mM Tris, pH 8.0, 100 mM acetyl CoA, 100 mM 5,5′-di-thiobis-(2-nitrobenzoic acid), 0.1% triton X-100, and 2–4 μg supernatant protein and was initiated with 100 μM oxaloacetate and monitored at 412 nm for 3 min at 25 °C.

2.5.2. Malate dehydrogenase activity

Malate dehydrogenase was measured as described by Kitto (1969). Aliquots (20 mg protein) were transferred into a medium containing 10 mM rotenone, 0.2% Triton X-100, 0.15 mM NADH, and 100 mM potassium phosphate buffer, pH 7.4, at 37 °C. The reaction was started by the addition of 0.33 mM oxaloacetate. Absorbance was monitored as described above.

2.5.3. Succinate dehydrogenase activity

Succinate dehydrogenase activity was determined according to the method of Fischer et al. (1985), and measured by following the decrease in absorbance due to the reduction of 2,6-di-chloro-indophenol (2,6-DCIP) at 600 nm with 700 nm as a reference wavelength ($\varepsilon = 19.1\ mM^{-1}\ cm^{-1}$) in the presence of phenazine methosulfate (PMS). The reaction mixture consisting of 40 mM potassium phosphate, pH 7.4, 16 mM succinate and 8 μM 2,6-DCIP was pre-incubated with 40–80 μg homogenate protein at 30 °C for 20 min. Subsequently, 4 mM sodium azide, 7 μM rotenone and 40 μM 2,6-DCIP were added and the reaction was initiated by the addition of 1 mM PMS and was monitored for 5 min.

2.6. Activities of mitochondrial respiratory chain enzymes

2.6.1. Complex I activity

NADH dehydrogenase (complex I) was evaluated according to Cassina and Radi (1996) by the determination of the rate of NADH-dependent ferricyanide reduction at $\lambda = 420$ nm.

2.6.2. Complex II activity

The activities of succinate-2,6-dichloroindophenol (DCIP)-oxidoreductase (complex II) were determined by the method described by Fischer et al.