Brain creatine kinase activity in an animal model of mania

Emilio L. Streck a,⁎, Graziela Amboni b, Giselli Scaini a, Priscila B. Di-Pietro a, Gislaine T. Rezin a, Samira S. Valvassori b, Gabrielle Luz b, Flávio Kapczinski c, João Quevedo b

a Laboratório de Fisiopatologia Experimental, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, 88806-000 Criciúma, SC, Brazil
b Laboratório de Neurociências, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, 88806-000 Criciúma, SC, Brazil
c Bipolar Disorders Program, Hospital de Clínicas de Porto Alegre, 90035-003 Porto Alegre, RS, Brazil
d Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, 90035-003 Porto Alegre, RS, Brazil

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Abstract

There is evidence pointing to dysfunction at the mitochondrial level as an important target for the understanding of the pathophysiology of bipolar disorder (BD). We assessed creatine kinase (CK) activity in rats submitted to an animal model of mania which included the use of lithium and valproate. In the acute treatment, amphetamine (AMPH) or saline was administered to rats for 14 days, and between day 8 and 14, rats were treated with either lithium, valproate or saline. In the maintenance treatment, rats were pretreated with lithium, valproate or saline, and between day 8 and 14, AMPH or saline were administered. In both experiments, locomotor activity was assessed by open-field test and CK activity was evaluated in hippocampus, striatum, cerebellum, whole cortex and prefrontal cortex. Our results showed that mood stabilizers reversed AMPH-induced behavioral effects. Moreover, AMPH (acute treatment) inhibited CK activity in hippocampus, striatum and cortex, but not in cerebellum and prefrontal cortex, and administration of lithium or valproate did not reverse the enzyme inhibition. In the maintenance treatment, AMPH decreased CK activity in saline-pretreated rats in hippocampus, striatum and cortex, but not in cerebellum and prefrontal cortex. AMPH administration in lithium- or valproate-pretreated animals decreased CK activity in hippocampus, striatum and cortex. Our results showed that AMPH inhibited CK activity and that mood stabilizers were not able to reverse and/or prevent the enzyme inhibition. These findings reinforce the hypothesis that mitochondrial dysfunction plays an important role in the pathophysiology of BD.

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Introduction

Bipolar disorder (BD) is a common and severe mood disorder associated with higher rates of suicide and disability (Belmaker, 2004; Kupfer, 2005), clinically characterized by the presence of manic symptoms (American Psychiatric Association, 1994; Belmaker, 2004). An adequate animal model of BD should resemble some features of a manic episode such as euphoria, irritability, aggressiveness, hyperactivity, insomnia or increased sexual drive. Considering the difficulty of modeling the highly complex mood swinging nature of BD, the psychostimulant-induced hyperactivity is the best established animal model of mania (Machado-Vieira et al., 2004). It has long been recognized that the administration of amphetamine (AMPH) induces manic symptoms in both normal human volunteers (Strakowski and Sax, 1998) and BD patients (Anand et al., 2000). The pharmacological management of BD includes the treatment of acute states and maintenance treatment in order to prevent new episodes. Mood stabilizing drugs, particularly lithium and valproate, are considered first line agents for both acute mania and maintenance treatment (Yatham et al., 2005). Several studies have suggested that the neuroprotective effects of lithium and valproate may be responsible for their therapeutical effects (Chuang et al., 2002; Li et al., 2002).

The pathophysiology of BD remains poorly understood, and recent studies have demonstrated that changes in intracellular pathways that regulate neuronal transmission, plasticity and
survival are associated with the pathophysiology of BD (Manji et al., 2001; Bezchlibnyk and Young, 2002; Coyle and Duman, 2003). Previous studies from our laboratory also showed that repeated administration of AMPH was associated with increased protein and lipid oxidative damage (Frey et al., 2006a) and an imbalance between superoxide dismutase and catalase activities in the prefrontal cortex, hippocampus and striatum (Frey et al., 2006b) in rat brain.

Creatine kinase (CK; E.C. 2.7.3.2) plays a central role in the metabolism of high-energy consuming tissues such as brain, skeletal muscle and heart, where it functions as an effective buffering system of cellular ATP levels (Bessman and Carpenter, 1985; Schnyder et al., 1991; Wallimann et al., 1992). It has been widely shown that a decrease in CK activity is associated with a neurodegenerative pathway that results in neuronal loss following brain ischemia (Tomimoto et al., 1993), neurodegenerative diseases (David et al., 1998; Aksenov et al., 2000) and other pathological states (Hamman et al., 1995; Gross et al., 1996).

Evidence from the literature also strongly indicate that metabolism impairment and mitochondrial dysfunction may be involved in pathophysiology of neuropsychiatric disorders, such as BD (Hough and Chuang, 2000; Kato and Kato, 2000; Konradi et al., 2004). In this context, we have recently shown that citrate synthase activity was inhibited in hippocampus of rats submitted to AMPH administration and that valproate, but not lithium, was able to prevent and reverse the enzyme inhibition caused by AMPH (Corrêa et al., 2007). Moreover, in vivo magnetic resonance spectroscopy studies have demonstrated a decrease in both pH and high-energy phosphates, such as phosphocreatine and ATP, in the frontal and temporal lobes of BD subjects (Kato et al., 1993, 1998; Deicken et al., 1995; Dager et al., 2004). Changes in brain compounds related to oxidative phosphorylation, energy production, and phospholipid metabolism have also been observed in BD patients (Stork and Renshaw, 2005). More recently, postmortem studies in hippocampus (Konradi et al., 2004) and cortex (Sun et al., 2006) of BD patients showed a decrease in the expression of nuclear genes coding for mRNAs of the mitochondrial respiratory chain enzymes. Finally, MacDonald et al. (2006) also presented very interesting results, showing that levels of CK mRNA are decreased in BD patients, especially in the hippocampus.

Therefore, considering that CK plays an important role in brain energy metabolism and that metabolism impairment and mitochondrial dysfunction may be involved in the pathophysiology of BD, the aim of this study was to investigate the effects of AMPH and mood stabilizers on CK activity in hippocampus, striatum, and prefrontal cortex of rats.

Methods

Animals

Adult male Wistar rats (250–300 g) were obtained from the Central Animal House of Universidade do Extremo Sul Catarinense. They were caged in groups of five with free access to food and water, and were maintained on a 12-h light–dark cycle (lights on at 7:00 am), at a temperature of 23 °C±1 °C. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care, with the approval of the Ethics Committee from Universidade do Extremo Sul Catarinense.

Acute treatment

The first set of experiments was designed in order to reproduce the management of an acute manic episode (reversal treatment). Animals received one daily i.p. injection of either AMPH (Sigma, St Louis, USA) 2 mg/kg or saline for 14 days. Between the 8th and the 14th day, saline- and AMPH animals were divided in three experimental groups: lithium, valproate and saline treatments. Lithium-treated animals received lithium 47.5 mg/kg i.p. twice a day, and valproate-treated animals received valproate 200 mg/kg i.p. twice a day. We have previously found that lithium 47.5 mg/kg i.p. and valproate 200 mg/kg i.p. per se did not alter locomotor behavior in male Wistar rats (Frey et al., 2006a,b,c). Locomotor activity was measured 2 h after the last injection, and the rats were killed right after the open-field task.

Maintenance treatment

The second set of experiments was designed to mimic the maintenance phase of BD treatment (prevention treatment). Animals received either lithium 47.5 mg/kg i.p. twice a day, valproate 200 mg/kg i.p. twice a day or saline for 14 days. Between the 8th and the 14th day, lithium-, valproate- and saline-treated animals were divided in two experimental groups: each treated group received one daily i.p. injection of either AMPH 2 mg/kg or saline. Locomotor activity was measured 2 h after the last injection, and the rats were killed right after the open-field task.

Locomotor activity

The locomotor activity was assessed using the open-field task. The task was performed in a 40×60 cm open field surrounded by 50 cm high walls made of brown polywood with a frontal glass wall. The floor of the open field was divided into 12 equal rectangles by black lines. The animals were gently placed on the left rear quadrant, in order to explore the arena for 5 min. Crossings of the black lines and rearings were counted.

Sample preparation

Hippocampus, striatum, cerebellum, cortex and prefrontal cortex were homogenized (1:20) in SETH buffer (0.32 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4). The homogenate was collected for determination of CK activity. Protein content was determined by the method described by Lowry et al. (1951) using bovine serum albumin as standard.
Creatine kinase activity

CK activity was measured in brain homogenates pretreated with 0.625 mM lauryl maltoside. The reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7 mM phosphocreatine, 9 mM MgSO₄ and approximately 0.4–1.2 μg protein in a final volume of 100 μL. After 15 min of pre-incubation at 37 °C, the reaction was started by the addition of 3.2 mmol of ADP plus 0.8 mmol of reduced glutathione. The reaction was stopped after 10 min by the addition of 1 μmol of p-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (1962). The color was developed by the addition of 100 μL 2% α-naphtol and 100 μL 0.05% diacetyl in a final volume of 1 mL and read spectrophotometrically after 20 min at 540 nm. Results were expressed as units/min × mg protein.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test when F was significant and are expressed as mean±standard deviation. All analyses were performed using the Statistical Package for the Social Science (SPSS) software.

Results

In the reversal treatment, AMPH increased locomotor activity in saline-treated rats and both lithium and valproate reversed AMPH-related hyperactive behavior. The administration of lithium or valproate in saline-treated animals did not change behavioral measures, indicating that the effects of mood stabilizers in AMPH-treated rats were not associated with sedation (F(5,24) = 4.71; p < 0.05) (Fig. 1). AMPH administration significantly inhibited CK activity in rat hippocampus, striatum and cortex, but not in cerebellum and prefrontal cortex. The administration of lithium and valproate in AMPH-treated animals did not reverse the inhibition of the enzyme in hippocampus, striatum and cortex (F(5,24) = 12.18; p < 0.01) (Fig. 2). In the prevention experiment, both lithium and valproate pretreatment were able to prevent AMPH-related hyperactivity. Saline administration in mood stabilizers-
pretreated animals demonstrated no behavioral effects ($F(5,24)=3.98; p<0.05$) (Fig. 3). Pretreatment with lithium and valproate in saline-treated animals did not change CK activity in any brain structure studied in this work. However, AMPH decreased the enzyme activity in hippocampus, striatum and cortex, but not in cerebellum and prefrontal cortex of saline-pretreated rats. Again, lithium and valproate pretreatment did not prevent the inhibition of CK activity ($F(5,24)=15.33; p<0.01$) (Fig. 4). All lithium-treated animals had lithium serum levels between 0.6 and 1.2 mEq/L, as recommended for the treatment of BD patients. Moreover, the regimen of valproate administration produces plasma concentrations around 30 μg/ml (Chang et al., 2001), comparable to mean concentrations of 32.5 μg/ml, effective in BD (Jacobsen, 1993).

Discussion

In the present study, we verified that 7 and 14 days of AMPH administration inhibited CK activity in the hippocampus, striatum, and cerebral cortex of rats. The creatine/phosphocreatine/CK system is important for normal energy homeostasis (Khuchua et al., 1998; Schlattner and Wallimann, 2000) by exerting several integrated functions, such as temporary energy buffering, metabolic capacity, energy transfer and metabolic control (Saks et al., 1985). The brain of adult rats, like other tissues with high and variable rates of ATP metabolism, presents high phosphocreatine concentration and CK activity (Khuchua et al., 1998; Schlattner and Wallimann, 2000). In the present work we studied the effects of the mood stabilizers lithium and valproate on CK activity in an animal model of mania.

In reversal and prevention models, the administration of lithium or valproate alone did not affect CK activity. In addition, lithium and valproate did not reverse or prevent CK activity inhibition caused by AMPH administration. Our results suggest that the mood stabilizing effects are not related to CK modulation. Because we used brain homogenates in the present study we cannot rule out that these mood stabilizers exert more localized effects on CK activity. Studies addressing isolated mitochondria or submitochondrial particles could help clarify this issue.

Fig. 3. Open-field test after seven days of treatment with mood stabilizers plus seven days of mood stabilizers and amphetamine. Data were analyzed by one-way analysis of variance followed by Tukey test when $F$ was significant. Values are expressed as mean±S.D., for ten animals in each group. * Different from control (saline plus saline); $p<0.05$.

Fig. 4. Creatine kinase activity after seven days of treatment with mood stabilizers plus seven days of mood stabilizers and amphetamine. Data were analyzed by one-way analysis of variance followed by Tukey test when $F$ was significant. Values are expressed as units per minute per mg protein, for six independent experiments performed in duplicate. * Different from control (saline plus saline); $p<0.01$. 
Some studies suggested that metabolism impairment and mitochondrial dysfunction are probably involved in pathophysiology of BD. We reported recently that citrate synthesize, an important enzyme of Krebs cycle, is inhibited by AMPH administration (Corrêa et al., 2007). Other studies showed that high-energy phosphates compounds, such as phospho-creatine and ATP, are decreased in brain of BD patients (Kato et al., 1993, 1998; Deicken et al., 1995; Dager et al., 2004). Postmortem studies in brain of BD patients also showed decreased levels of mRNA for mitochondrial respiratory chain enzymes (Konradi et al., 2004; Sun et al., 2006) and CK (MacDonald et al., 2006). In this context, it is known that a diminution of CK activity may potentially impair energy homeostasis, contributing to brain damage. Besides, CK inhibition has also been observed in neurodegenerative and mental diseases, such as Alzheimer’s disease, schizophrenia (Burbaea et al., 2003) and animal models of some inborn errors of metabolism affecting the brain (Zugno et al., 2006).

Taking together our present findings and those from MacDonald et al. (2006), CK may be inhibited and/or downregulated in BD. The inhibition of CK activity by AMPH reinforces the hypothesis that metabolism impairment is involved in the pathophysiology of BD. It is well known that abnormalities in respiratory chain complexes activities and ATP synthesis lead to cellular degeneration (Calabrese et al., 2001). We also showed that lithium and valproate had no effect on the enzyme inhibition caused by AMPH. However, Gandarias et al. (1989) showed that CK is increased in hippocampus and hypothalamus after acute lithium administration. In the present paper we used a different protocol, whereas lithium was chronically administered.

It is important to reinforce that these drugs present other pharmacological effects and are important in the treatment of BD patients. In this context, some studies showed that AMPH induces changes in various systems, such as signaling pathways and kinases and phosphatases activities, and that mood stabilizers prevent and/or reverse some of these biochemical and other behavioral effects caused by amphetamine (Gould and Manji, 2002; Mai et al., 2002; Einat et al., 2003; Beaulieu et al., 2004; Bell et al., 2005). From our findings, we suggest that neuroprotective pathways induced by mood stabilizers may not involve this specific (CK activity) biochemical effect.

The mechanisms underlying the pathophysiology of BD are still not fully understood. However, evidence suggest that energy impairment is involved in BD. If the inhibition on CK also occurs in BD patients, it is tempting to speculate that the reduction of brain metabolism may be probably related to the pathophysiology of this disease.

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