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Effects of tamoxifen on tricarboxylic acid cycle enzymes in the brain of rats submitted to an animal model of mania induced by amphetamine

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ABSTRACT

The neurobiological basis of bipolar disorder (BD) remains unknown; nevertheless, mitochondrial dysfunction has been identified in this disorder. Inactivation of any step in the tricarboxylic acid (TCA) cycle can impair mitochondrial ATP production. There is recent evidence indicating that PKC is an important therapeutic target for bipolar disorder. Therefore, we evaluated the effects of tamoxifen (TMX —a PKC inhibitor) on the activities of enzymes in the TCA cycle of rat brains subjected to an animal model of mania induced by amphetamine. In the reversal treatment, Wistar rats were first treated with d-AMPH or saliratsne (Sal) for 14 days. Thereafter, between days 8 and 14, the rats were administered TMX or Sal. The citrate synthase, succinate dehydrogenase, and malate dehydrogenase were evaluated in the frontal cortex, hippocampus, and striatum. The d-AMPH administration inhibited TCA cycle enzymes activity in all analyzed structures, and TMX reversed d-AMPH-induced dysfunction. In addition, we observed a negative correlation between d-AMPH-induced hyperactivity and the activity of these enzymes in the rat's brain. These findings suggested that TCA cycle enzymes inhibition can be an important link for the mitochondrial dysfunction seen in BD, and TMX exert protective effects against the d-AMPH-induced TCA cycle enzymes dysfunction.

1. Introduction

Although the neurobiological basis of bipolar disorder (BD) remains unknown, mitochondrial dysfunction, oxidative stress, and oxidative cell damage have been identified in this disorder (Clay et al., 2011; Gigante et al., 2011). Mitochondria play a key role in the production of the cell energy. The cerebral oxygen consumption and ATP production are coupled to the tricarboxylic acid (TCA) cycle, therefore, inactivation of any TCA step can alter mitochondrial ATP production (Blass and Brown, 2000).

In the first step of the TCA cycle, citrate synthase (CS) catalyzes the condensation of acetyl-CoA with oxaloacetate to form citrate, a tricarboxylic acid from which a common name of the cycle has been derived (Wiegand and Remington, 1986). Succinate is oxidized to fumarate by the membrane-linked succinate dehydrogenase (SDH) complex. SDH is the only TCA cycle enzyme directly linked to the electron transfer chain, because it is part of complex II and forms the peripheral membrane of this complex (Oyedotun and Lemire, 2004; Kantorovich and Pacak, 2010). The final reaction, catalyzed by malate dehydrogenase (MDH), constitutes the last oxidation step. Malate dehydrogenase (MDH) catalyzes the conversion of oxaloacetate and malate, utilizing the NAD/NADH coenzyme system (Minard and McAlister-Henn, 1991).

The effects of stimulants, such as amphetamine, on behavior have been widely used as an animal model of mania, because it induces psychomotor agitation, which is commonly observed during mania, and locomotor activity is easily measured in rats (Davies et al., 1974; Berggren et al., 1978). Contemplating the construct validity of the model, changes in the dopaminergic system have been shown to be the predominant etiologic factor for BD (Berk et al., 2007; Valvassori et al., 2010). In addition, studies have suggested that dopamine (DA) can be an important link for the mitochondrial dysfunction seen in BD (Greene, 2006; Valvassori et al., 2010). The DA systems implicated in the pathophysiology of mood disorders are the nigrostriatal and the mesocorticolimbic circuits, which innervates the striatum, amygdala, ventral hippocampus, and frontal cortex. These cerebral structures are related to a variety of behavioral functions, such as motivation and reward that are impaired in BD (Kalivas et al., 1993).

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Recently, it was demonstrated that methamphetamine-induced neurotoxicity in dopaminergic neuronal cells is modulated by protein kinase C (PKC) (Lin et al., 2012). Lithium (Li) and Valproic acid (VPA), drugs used for treatment and prophylaxis of BD, have been characterized as a PKC inhibitor (Zarate and Manji, 2006). In addition, several clinical studies have suggested possible efficacy of tamoxifen (TMX—a PKC inhibitor) in mania (Bebchuk et al., 2000; Kulkarni et al., 2006; Zarate et al., 2007; Yildiz et al., 2008).

Thus, we evaluated the effects of TMX on the activities in mitochondrial enzymes of the TCA cycle in the frontal, hippocampus and striatum from rats submitted to an animal model of mania induced by d-AMPH.

2. Methods

2.1. Experimental methods

In the present study, we have extended the investigation of the effects of TMX on d-AMPH induced neurochemical alterations in an animal model of mania by measuring the activities of mitochondrial enzymes of the Krebs cycle in frontal, hippocampal, and striatal samples that were kept frozen at −70 °C from one of our previous experiments (Steckert et al., 2012). The detailed description of the experiments has been published elsewhere (Steckert et al., 2012); therefore, we summarize here the treatment regimens and describe the subsequent steps performed for the present investigation.

2.2. Animals

The subjects were adult male Wistar rats (weighting 250–350 g) obtained from our breeding colony. 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.

2.3. Drugs and pharmacological procedures

Rats received intraperitoneal (i.p.) injection of either d-AMPH (2 mg/kg) or Sal once a day for a period of 14 days. From the 8th to the 14th day (treatment for 7 days), d-AMPH and Sal-treated animals also received Sal (i.p.—twice a day) or TMX (1 mg/kg i.p.—twice a day), totaling four experimental groups of five animals per group: Sal+Sal, Sal+TMX, d-AMPH+Sal and d-AMPH+TMX. No behavioral assessment was performed between days 1 and 14. On the 15th day of treatment, the animals received a single injection of d-AMPH or Sal and, 2 h after, they were killed by decapitation and frontal cortex, hippocampus, and striatum were manually dissected on ice, rapidly frozen on dry ice and stored at −70 °C until assayed. The brain structures were dissected by trained researchers based on the stereotaxic atlas from Paxinos and Watson (1986).

Note: The range of doses of TMX employed in this work was chosen based on our previous study (Steckert et al., 2012).

2.4. Behavioral assessment

We used the open field task to assess locomotor activity. The task was performed in a 40 × 60 cm^2 open field surrounded by 50 cm-high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into nine equal rectangles with black lines. The animals were gently placed on the left rear rectangle and were allowed to explore the arena. Crossings of the black lines were counted for 5 min. The rats were sacrificed by decapitation right after the last open-field evaluation and the frontal cortex, hippocampus and striatum were dissected, rapidly frozen, and stored at −70 °C until assayed.

Note: This analysis was made in previous study from our research group (Steckert et al., 2012). The data from five animals was used to correlate behavioral and biochemical data through Pearson's correlation. It is important emphasizes that biochemical data are original and was obtained in the present study.

2.5. Activities of enzymes of Krebs cycle

Citrate synthase activity

Citrate synthase activity was assayed according to the method described by Shepherd and Garland (1969). 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.

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.

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 (ε = 19.1 mM^{-1} cm^{-1}) in the presence of phenazine methasulphate (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. Statistical analysis

Data were analyzed by two-way analysis of variance followed by Tukey's test when F was significant and are expressed as mean ± S.E.M. Correlations were analyzed using the Pearson correlation test. All analyses were performed using the Statistical Package for the Social Science (SPSS; version 16.0) software.

3. Results

Results for CS activity are shown in Fig. 1A. CS activity was significantly decreased in all brain structures evaluated in d-AMPH+Sal group as compared to the control (Sal+Sal) group. This reduction was significantly reversed by the treatment with TMX (d-AMPH+TMX). TMX treatment in the control group (Sal+TMX) did not modify the enzyme activity.

Data from the two-way ANOVA for d-AMPH administration [frontal cortex: $F(1.12)=17.92$, $p=0.001$; hippocampus: $F(1.12)=18.98$, $p<0.001$; striatum: $F(1.12)=18.24$, $p=0.001$], treatment [frontal cortex: $F(1.12)=35.46$, $p<0.001$; hippocampus: $F(1.12)=20.42$, $p<0.001$; striatum: $F(1.12)=20.33$, $p<0.001$], and d-AMPH administration × treatment interaction [frontal cortex: $F(1.12)=26.96$, $p<0.001$; hippocampus: $F(1.12)=9.19$, $p=0.01$; striatum: $F(1.12)=20.87$, $p<0.001$].

As depicted in Fig. 1B. Administration of d-AMPH resulted in a marked inhibition of SDH activity in the hippocampus, striatum, and frontal cortex in the d-AMPH+Sal group. TMX reversed d-AMPH-induced inhibition of SDH activity in all brain regions analyzed (d-AMPH+TMX). TMX treatment in the control group (Sal+TMX) did not modify the enzyme activity.

Data from the two-way ANOVA for d-AMPH administration [frontal cortex: $F(1.12)=40.2$, $p<0.001$; hippocampus: $F(1.12)=64.61$, $p<0.001$; striatum: $F(1.12)=35.09$, $p<0.001$], treatment [frontal cortex: $F(1.12)=10.96$, $p=0.006$; hippocampus: $F(1.12)=21.47$, $p<0.001$; striatum: $F(1.12)=14.48$, $p<0.001$], and d-AMPH administration × treatment interaction [frontal cortex: $F(1.12)=7.35$, $p=0.01$; hippocampus: $F(1.12)=7.16$, $p=0.02$; striatum: $F(1.12)=11.12$, $p=0.005$].

Results for MDH activity are shown in Fig. 1C. Administration of d-AMPH resulted in a marked inhibition of MDH activity in the hippocampus, striatum and frontal cortex in the d-AMPH+Sal group. TMX reverses d-AMPH-induced inhibition in the hippocampus, and partially in the striatum and frontal cortex (d-AMPH+TMX). Once more, the TMX regime in the control group (Sal+TMX) did not modify the complex's activity.

Data from the two-way ANOVA for d-AMPH administration [frontal cortex: $F(1.12)=139.2$, $p<0.001$; hippocampus: $F(1.12)=3.8$, $p=0.074$; striatum: $F(1.12)=136.57$, $p<0.001$], treatment [frontal cortex: $F(1.12)=20.47$, $p<0.001$; hippocampus: $F(1.12)=$