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Mitochondrial respiratory chain activity in an animal model of mania induced by ouabain

Freitas TP, Rezin GT, Fraga DB, Moretti M, Vieira JS, Gomes LM, Borges LS, Valvassori SS, Quevedo J, Streck EL. Mitochondrial respiratory chain activity in an animal model of mania induced by ouabain.

Objectives: Bipolar disorder (BD) is a mental illness associated with higher rates of suicide. The present study aims to investigate the brain mitochondrial respiratory chain activity in an animal model of mania induced by ouabain.
Methods: Adult male Wistar rats received a single intracerebroventricular administration of ouabain (10^{-3} and 10^{-2} M) or vehicle. Locomotor activity was measured using the open field test. Mitochondrial respiratory chain activity was measured in the brain of rats 1 h and 7 days after ouabain administration.
Results: Our results showed that spontaneous locomotion was increased 1 h and 7 days after ouabain administration. Complexes I, III and IV activities were increased in the prefrontal cortex, hippocampus and striatum immediately after the administration of ouabain, at the concentration of 10^{-3} and 10^{-2} M. Moreover, complex II activity was increased only in the prefrontal cortex at the concentration of 10^{-2} M. On the other hand, no significant alterations were observed in complex I activity 7 days after ouabain administration. However, an increase in complexes II, III and IV activities was observed only in the prefrontal cortex at the concentration of 10^{-2} M.
Conclusion: Our findings suggest an increase in the activities of mitochondrial respiratory chain in this model of mania. A possible explanation is that these findings occur as a rebound effect trying to compensate for a decrease of ATP deprivation in BD. The present findings suggest that this model may present good face validity and a limitation in construct validity.

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Introduction

Bipolar disorder (BD) is among the 10 most disabling medical conditions in the world (1), and it has been associated with increased risk of morbidity, mortality and psychiatric comorbidity (2,3). BD is a severe mood disorder with unclear pathophysiology and uncertain pathogenesis. The key factor of the condition is a manic episode. Mania is characterised by an elated or irritable mood, reduced need for sleep, psychomotor activation and excessive involvement in potentially problematic behaviour (4).

The availability of an animal model would accelerate BD research by improving the understanding of the pathophysiology of the disorder and providing the possibility of preclinical pharmacological screening (5). In seeking a reliable animal model at least three criteria must be met: face, construct and predictive validities. Face validity represents how similar the model can mimic the symptoms of a determinate human disorder, whereas construct validity refers to commonalties between the mechanism of the model and of the human disorder. Finally, the predictive validity refers to the efficacy of treatment drugs use for human disease for the phenotype of the model animal (6).

The intracerebroventricular (i.c.v) administration of ouabain (a Na^+, K^+-ATPase inhibitor) in rats has

been suggested as an animal model of BD. Ouabain induces hyperactivity and mimics some symptoms of human bipolar mania (face validity), which was normalised by the treatment with lithium and haloperidol (predictive validity) (5,7,8). It has also been shown that Na^+, K^+-ATPase is altered in psychiatric disorders such as BD (8–10). Additionally, the sodium pump activity is reduced in manic and depressed bipolar patients (11), pointing to the potential constructs validity of this animal model of mania induced by ouabain. The decrease in the activity of Na^+, K^+-ATPase could be because of a reduction in ATP synthesis or to an increased production of inhibitors. This could be an important link in the pathological response to deficiencies in energy metabolism (12). However, the biochemical mechanisms underlying the development of the alterations caused by the Na^+, K^+-ATPase inhibitors remain to be clarified.

Many lines of evidence suggest that mitochondria play a central role in BD (13–15). Mitochondrial oxidative phosphorylation is the major ATP-producing pathway (16). Energy, in the form of ATP, is obtained in the mitochondria through a series of reactions in which electrons liberated from reducing substrates, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH), are delivered to O_2 through a chain of respiratory proton pumps (17). An abnormal cellular energy state can lead to alteration in neuronal function, plasticity and brain circuits, and thereby to the cognitive and mood alteration characteristic of BD (18,19).

Based on the hypothesis that mitochondrial dysfunction might be involved in the pathophysiology of BD, in the present work we evaluated the activities of mitochondrial respiratory chain complexes in the brain of rats after ouabain administration.

Methods

Animals

Adult male Wistar rats (250–300 g) obtained from Central Animal House of Universidade do Extremo Sul Catarinense were housed in groups of five with free access to food and water and maintained on a 12-h light-dark cycle (lights on 7:00 am) at a temperature of $22 \pm 1\ ^{\circ}C$. 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 local Ethics Committee.

Surgical procedure and treatment

Animals were intraperitoneally anaesthetised with ketamine (80 mg/kg) and xylasine (10 mg/kg). In a stereotaxic apparatus, the skin of the rat skull was removed and a 27-gauge 9-mm guide cannula was placed at 0.9 mm posterior to bregma, 1.5 mm right from the midline and 1.0 mm above the lateral brain ventricle. Through a 2-mm hole made at the cranial bone, a cannula was implanted 2.6 mm ventral to the superior surface of the skull, and fixed with jeweller acrylic cement. Animals were tested on the third day following surgery. A 30-gauge cannula was fitted into the guide cannula and connected by a polyethylene tube to a microsyringe. The tip of the infusion cannula protruded 1.0 mm beyond the guide cannula aiming the right lateral brain ventricle. Each animal received 5 µl of vehicle (NaCl 0.9%) or ouabain (10^{-3} and 10^{-2} M; Sigma Chemical Co., Saint Louis, MO, USA) over 30 s. Immediately after the injection every rat was placed individually into the open field apparatus.

Behavioural assessment

The open field task was used in order to assess spontaneous locomotor activity. The task was performed in a 40×60 cm 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 by black lines. The animals were gently placed on the left rear rectangle and were allowed to explore the arena. The number of crossings of the black lines and rearings were counted during 5 min. Animals were observed in the open field four times, in order to induce habituation to novelty: (session 1) before the cannula implantation (0 h); (session 2) on the third day following surgery (72 h) and (session 3) on the fourth day following surgery (96 h), immediately after i.c.v. injection of ouabain or saline. A separate group of rats was also submitted 7 days after the i.c.v. injection of ouabain or saline (session 4).

Sample preparation

The rats were killed immediately after the open field task and 7 days after i.c.v ouabain administration, the hippocampus, striatum and prefrontal cortex were dissected and homogenised (1:20) in SETH buffer (0.32 M sucrose, 1 mM EDTA, 10 mM Tris–HCl, pH 7.4). The homogenate was collected to determine the activity of mitochondrial respiratory chain enzymes. Protein content was determined by the method described by Lowry et al. (20) using bovine serum albumin as standard.

Activities of mitochondrial respiratory chain enzymes

NADH dehydrogenase (complex I) was evaluated by the method described by Cassina and Radi (21)

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by the rate of NADH-dependent ferricyanide reduction at $\lambda = 420$ nm. The activities of succinate-2, 6-dichloroindophenol (DCIP)-oxidoreductase (complex II) and succinate:cytochrome *c* oxidoreductase (complex II-III) were determined by the method described by Fischer et al. (22). Complex II activity was measured by following the decrease in absorbance because of the reduction of DCIP at $\lambda = 600$ nm. Complex II-III activity was measured by cytochrome *c* reduction from succinate at $\lambda = 550$ nm. The activity of cytochrome *c* oxidase (complex IV) was assayed according to the method described by Rustin et al. (23), measured by following the decrease in absorbance because of the oxidation of previously reduced cytochrome *c* at $\lambda = 550$ nm. The activities of the mitochondrial respiratory chain complexes were calculated as nmol/min mg protein.

Statistical analysis

All data are presented as mean and standard deviation of the mean. Differences among experimental groups were determined by one-way analysis of variance (ANOVA) followed by the Tukey test when *F* was significant. In all comparisons, statistical significance was set at $p < 0.05$.

Results

As illustrated in Fig. 1, the spontaneous locomotion of rats submitted to the open field apparatus during the first and second sessions was not different among groups. However, the i.c.v. administration of ouabain at 10^{-3} and 10^{-2} M increased rat spontaneous locomotion when compared to control group immediately after injection. Hyperlocomotion was still observed 7 days following a single i.c.v. injection of ouabain 10^{-3} and 10^{-2} M.

As seen in Figs 2 and 3, a significant increase in complexes I, II-III and IV activities was detected in the prefrontal cortex, hippocampus and striatum immediately after the administration of ouabain, at the concentration of 10^{-3} and 10^{-2} M. Moreover, complex II activity was increased only in the prefrontal cortex at the concentration of 10^{-2} M (Fig. 2). On the other hand, in animals which complexes activities were assessed 7 days following ouabain administration, no significant alterations were observed in complex I activity in the prefrontal cortex, hippocampus and striatum. However, an increase in complexes II, II-III and IV activities in the prefrontal cortex was observed after 10^{-2} M ouabain administration. The complexes II, II-III and IV activities in the hippocampus and striatum at the concentration of 10^{-3} M were not altered (Fig. 3).

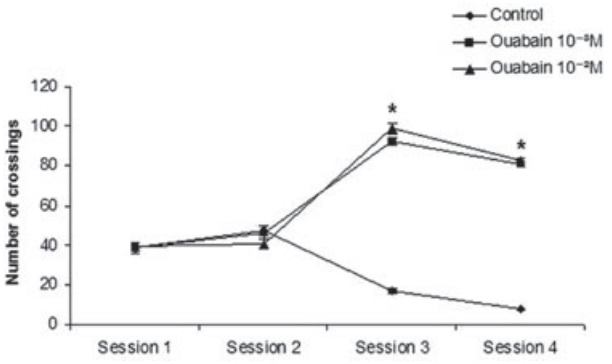

Fig. 1. Effects of the i.c.v. administration of ouabain (10^{-3} and 10^{-2} M) or saline on number of crossings in rats subjected to the open field test for 5 min. Locomotor activity was assessed in the open field test for four times: before surgery (session 1), on the third day following surgery (session 2), immediately after i.c.v. administration of ouabain or saline (session 3) and 7 days after i.c.v. administration of ouabain (session 4). $*p < 0.05$ versus saline group, according to ANOVA followed by the Tukey test ($n = 12$).

Discussion

In the present study we observed the spontaneous locomotion of rats 1 h and 7 days after ouabain administration. We also evaluated the activities of mitochondrial respiratory chain complexes I, II, II-III and IV 1 h and 7 days after ouabain administration at the concentration of 10^{-3} and 10^{-2} M. Our results showed that a single i.c.v. injection of ouabain induced hyperlocomotion in rats, which remains up to 7 days after its administration. These findings are in complete agreement with literature which showed stimulatory effect for ouabain in rats immediately after the injection (5,7,24) and also 9 days after a single injection of 10^{-3} M ouabain (25). As previously shown, these observations strength the view that ouabain-induced mania-like behaviour in rats could be a useful model to investigate some aspects linked to the chronicity of BD, such as long-term neurochemical alterations (26).

Ouabain is a Na^+, K^+-ATPase inhibitor, a membrane enzyme responsible for the generation of the membrane potential through the active transport of sodium and potassium ions in the central nervous system necessary to maintain neural activity, neurotransmitter release and animal behaviour (27–29). It is present at high concentrations in the brain, consuming about 40–50% of the ATP obtained in this tissue (30). Accumulating evidence has strengthened the possible involvement of Na^+, K^+-ATPase dysfunction in the pathophysiology of BD (31,32). A study showed that sodium pump activity is decreased in acute mania compared to recovered euthymic bipolar individuals (33). These observations suggest the ouabain-induced mania-like behaviour in rats evokes

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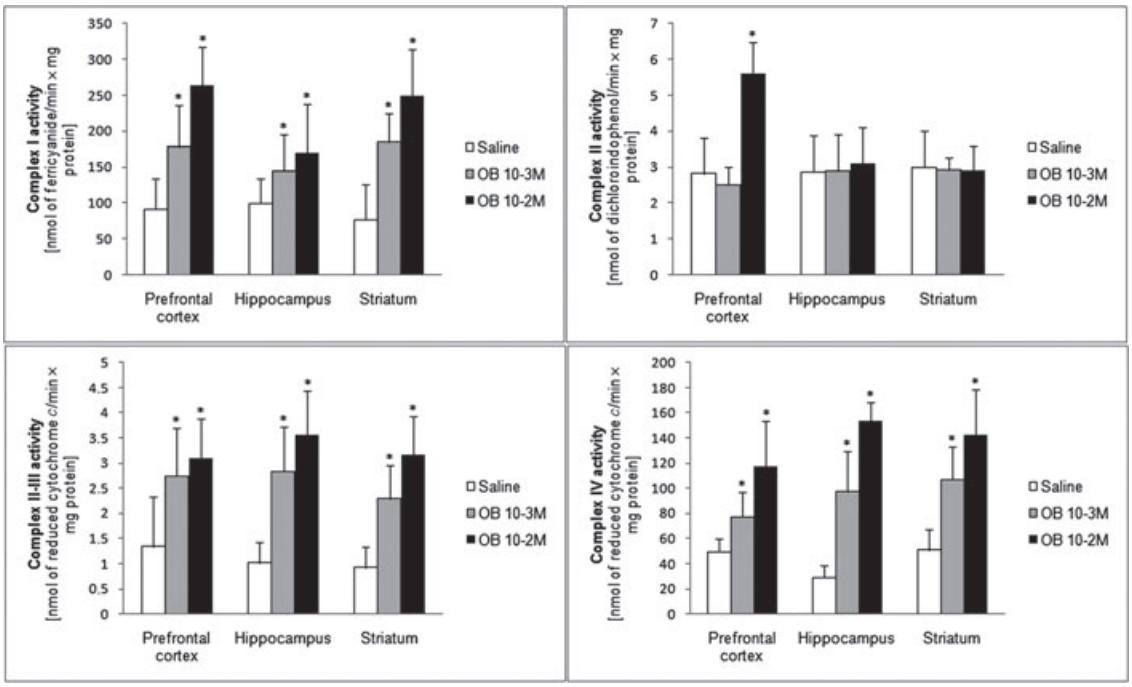

Fig. 2. Activities of complexes I, II, II-III and IV immediately after ouabain (10^{-3} and 10^{-2} M) or saline i.c.v. administration in the prefrontal cortex, hippocampus and striatum of rats. $^{*}p < 0.05$ versus saline group, according to ANOVA followed by the Tukey test. White squares: saline; grey squares: ouabain 10^{-3} M; black squares: ouabain 10^{-2} M ($n = 6$).

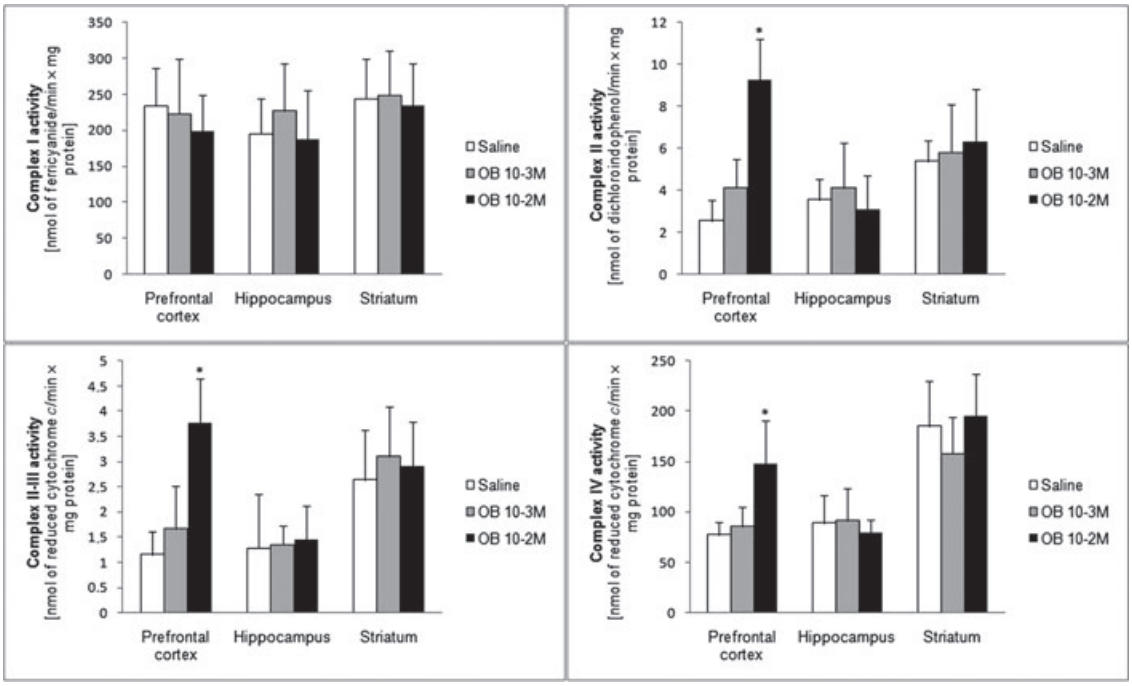

Fig. 3. Activities of complexes I, II, II-III and IV 7 days following ouabain (10^{-3} and 10^{-2} M) or saline i.c.v. administration in the prefrontal cortex, hippocampus and striatum of rats. $^{*}p < 0.05$ versus saline group, according to ANOVA followed by the Tukey test. White squares: saline; grey squares: ouabain 10^{-3} M; black squares: ouabain 10^{-2} M ($n = 6$).

some Na^+, K^+-ATPase dysfunction, similar to that reported in bipolar patients (34).

Besides the involvement of Na^+, K^+-ATPase in BD, studies also suggested that metabolism impairment may play a key role in the pathophysiology of BD (13,35–38). Corrêa et al. (39) showed that amphetamine administration inhibited citrate synthase activity in the brain of rats; citrate synthase

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is localised within cells in the mitochondrial matrix and catalyses the condensation of oxaloacetate and the acetyl group of acetyl coenzyme-A, the first step of Krebs cycle. In addition, citrate synthase activity has been used as a quantitative enzyme marker for the presence of intact mitochondria (40). Postmortem studies in the brain of BD patients also showed decreased levels of mRNA for mitochondrial respiratory chain enzymes (36,41) and creatine kinase (42). In this context, it has been recently shown that amphetamine administration inhibited creatine kinase activity in the brain of rats (38). Creatine kinase is an enzyme that catalyses the reversible transphosphorylation of creatine by ATP, plays a key role in cellular energy buffering and energy transport, particularly in cells with high and fluctuating energy requirements, including neurons (43). We have also recently showed that creatine kinase activity was inhibited immediately after the administration of ouabain in the striatum, hippocampus and prefrontal cortex. Moreover, the enzyme was not affected in the striatum and hippocampus 7 days after ouabain administration (26).

In the present work, we observed that complexes I, II, III and IV activities were increased in the brain of rats immediately after the administration of ouabain, which remains up to 7 days in some brain areas. Mitochondrial oxidative phosphorylation is the major ATP-producing pathway, which supplies more than 95% of the total energy requirement in the cells (44). It has been suggested that reduction in the Na^+, K^+-ATPase activity is secondary to the ATP depletion caused by suppression of oxidative phosphorylation (45). Others' studies suggest that hypoxia-induced suppression of the Na^+, K^+-ATPase function in different cell types is not necessarily linked to ATP deprivation (46–51). However, the reason why the activities of the mitochondrial respiratory chain complexes were increased in this animal model of mania induced by ouabain is not known, as most of the studies showed metabolism impairment in BD patients and animal models of mania. A possible explanation could be that the increase in the activities of mitochondrial respiratory chain complexes occurs as a rebound effect trying to compensate for a decrease of ATP deprivation in BD.

Moreover, the present findings suggest that this model may present good face validity (represents how similar the model can mimic the symptoms of a determinate human disorder), as the administration of ouabain increased rat spontaneous locomotion when compared to control group. However, this model may present a limitation in construct validity (refers to commonalties between the mechanism of the model and of the human disorder) only in the parameters evaluated in this study, the activity of mitochondrial respiratory chain complexes. More studies should be performed in order to better understand the mechanisms involving brain energy metabolism in this animal model of mania.

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