Acta Neuropsychiatrica

Acta Neuropsychiatrica 2012

DOI: 10.1111/j.1601-5215.2012.00650.x

In vitro effect of antipsychotics on brain energy metabolism parameters in the brain of rats

Scaini G, Rochi N, Morais MOS, Maggi DD, De-Nês BT, Quevedo J, Streck EL. *In vitro* effect of antipsychotics on brain energy metabolism parameters in the brain of rats.

Objective: Typical and atypical antipsychotic drugs have been shown to have different clinical, biochemical and behavioural profiles. It is well described that impairment of metabolism, especially in the mitochondria, leads to oxidative stress and neuronal death and has been implicated in the pathogenesis of a number of diseases in the brain. In this context, we investigated the *in vitro* effect of antipsychotic drugs on energy metabolism parameters in the brain of rats.
Methods: Clozapine (0.1, 0.5 and 1.0 mg/ml), olanzapine (0.1, 0.5 and 1.0 mg/ml) and aripiprazole (0.05, 0.15 and 0.3 mg/ml) were suspended in buffer and added to the reaction medium containing rat tissue homogenates and the respiratory chain complexes, succinate dehydrogenase and creatine kinase (CK) activities were evaluated.
Results: Our results showed that olanzapine and aripiprazole increased the activities of respiratory chain complexes. On the other hand, complex IV activity was inhibited by clozapine, olanzapine and aripiprazole. CK activity was increased by clozapine at 0.5 and 1.0 mg/ml in prefrontal cortex, cerebellum, striatum, hippocampus and posterior cortex of rats. Moreover, olanzapine and aripiprazole did not affect CK activity.
Conclusion: In this context, if the hypothesis that metabolism impairment is involved in the pathophysiology of neuropsychiatric disorders is correct and these results also occur *in vivo*, we suggest that olanzapine may reverse a possible diminution of metabolism.

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Keywords: aripiprazole; clozapine; creatine kinase; mitochondrial respiratory chain; olanzapine

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Accepted for publication December 28, 2011

Significant outcomes

- Olanzapine and aripiprazole increased the activities of respiratory chain complexes I, II and II–III.
- Complex IV activity was inhibited by clozapine, olanzapine and aripiprazole.
- Creatine kinase (CK) activity was increased by clozapine in prefrontal cortex, cerebellum, striatum, hippocampus and posterior cortex of rats.
- Olanzapine and aripiprazole did not affect CK activity.

Limitations

- It is necessary to investigate other important steps of energy metabolism.

Introduction

Neuropsychiatric disorders such as schizophrenia, depression and bipolar disorder have been related to dysfunction in the brain metabolism (1–3), and on neurotrophic factor expression (4–7), alterations in the neuronal function and survival (8,9), as well as abnormal synaptogenesis and neurotransmission (1,6,7). The metabolism dysfunction includes mitochondrial impairment (2,10), increase in reactive oxygen species (ROS) production and expression of biochemical markers of cellular degeneration (8,11–14).

Atypical antipsychotics such as clozapine and olanzapine exhibit serotonin/dopamine antagonistic properties associated with fewer extrapyramidal symptoms than conventional antipsychotics (9,15). Aripiprazole is an atypical neuroleptic that exhibits a high binding affinity for D_2 and D_3 receptors, a moderate affinity for D_4 receptors and a low affinity for D_1 receptors. Preclinical studies have also indicated that aripiprazole has a relatively high affinity for serotonin 5-HT_{2A} and 5-HT_{1A} receptors. It displays partial agonist activity at the 5-HT_{1A} receptor and antagonistic activity at the 5-HT_{2A} receptor (16). In humans, recent evidence suggests that schizophrenic patients treated with atypical antipsychotics may present a better performance in cognitive tasks when compared to patients treated with typical antipsychotics (9,17).

Mitochondria are intracellular organelles which play a crucial role in adenosine triphosphate (ATP) production (18). Mitochondrial oxidative phosphorylation is the major ATP-producing pathway (19). Energy, in the form of ATP, is obtained in the mitochondria through a series of reactions in which electrons liberated from reducing substrates NADH and $FADH_2$ are delivered to O_2 via the electron transport chain, which consists of four multimeric complexes (I, II, III and IV) plus two small electron carriers, coenzyme Q (or ubiquinone) and cytochrome *c*. The energy obtained by the reactions of the electron transport chain is used to pump protons from the mitochondrial matrix into the intermembrane space located between the inner and outer mitochondrial membranes. This process creates an electrochemical proton gradient, which is utilised by complex V (or ATP synthase) to catalyse the formation of ATP by the adenosine diphosphate (ADP) (20,21). Another way to produce ATP is through CK (EC 2.7.3.2) which is a crucial enzyme for high energy-consuming tissues like brain, skeletal muscle and heart. This enzyme works as a buffering system of cellular ATP levels, playing a central role in energy metabolism. CK catalyses the reversible transfer of the phosphoryl group from phosphocreatine to ADP, regenerating ATP (22,23). In addition, CK may be considered as a marker of creatine/phosphocreatine system functioning in brain (24). In this context, it has been widely shown diminution of CK activity may potentially impair energy homeostasis, contributing to cell death (25–29).

The brain contains a large number of mitochondria, being more susceptible to fluctuations on aerobic metabolism. Neurons have a high metabolic rate, limited stores of glucose or glycogen, low anaerobe capacity and a near exclusive dependence on glucose as an energy substrate (30). It is well described that impairment of energy production caused by mitochondrial dysfunction has been implicated in the pathogenesis of a number of diseases, including neurological conditions such as dementia, cerebral ischaemia, Alzheimer's disease and Parkinson's disease (12,29–34). Neuropsychiatry disorders such as schizophrenia, depression and bipolar disorder have also been related to dysfunction on neurotrophic factor expression (4–7), dysfunction in the brain metabolism (1,35) alterations in the neuronal function and survival (36,37) as well as abnormal synaptogenesis and neurotransmission (1,6,7). In addition, Assis and colleagues (38) have recently shown that brain CK activity is altered by antipsychotics. Moreover, succinate dehydrogenase activity was inhibited in cerebellum and striatum after olanzapine and clozapine administration, respectively, whereas aripiprazole increased the enzyme in prefrontal cortex (39). Some studies also reported that chronic exposure to clozapine resulted in significant changes in the activities of antioxidant enzymes and oxidative damage in rat brain, but no change was observed with olanzapine administration (40).

Considering that reduction of brain energy metabolism is related to neurological dysfunction and that some neuroleptics alter oxidative stress parameters and cognitive function, in this study we evaluated the effects *in vitro* of some antipsychotics, named clozapine, olanzapine and aripiprazole on mitochondrial respiratory chain and CK activities in brain (hippocampus, striatum, cerebellum, posterior cortex and prefrontal cortex) of rats.

Materials and methods

Animals

Adult male Wistar rats (250–300 g) were obtained from the Central Animal House of University of Extremo Sul Catarinense, Criciúma, SC, Brazil. 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 07:00 h), at a temperature of 22 ± 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

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and the Brazilian Society for Neuroscience and Behavior recommendations for animal care, with the approval of UNESC Ethics Committee.

Drugs

Clozapine was purchased from Novartis Biociências AS, São Paulo, Brazil (Leponex®). Olanzapine was provided from Eli Lilly do Brasil Ltda, São Paulo, Brazil (Zyprexa®). Aripiprazole was purchased from Bristol-Myers Squibb, São Paulo, Brazil (Abilify®).

Tissue and homogenate preparation

On the day of the experiments the animals were killed by decapitation ($n = 6$), the brain was removed and cerebellum, hippocampus, striatum, prefrontal cortex and posterior cortex were homogenised (1:10, w/v) in SETH buffer, pH 7.4 (250 mM sucrose, 2 mM ethylenediaminetetraacetic acid, 10 mM Trizma base, 50 IU/ml heparin). The homogenates were centrifuged at 800 × g for 10 min and the supernatants kept at −70 °C until used for enzymes activity determination. The maximal period between homogenate preparation and enzyme analysis was always less than 5 days. Protein content was determined by the method described by Lowry and colleagues (41) using bovine serum albumin as standard. Clozapine (0.1, 0.5 and 1.0 mg/ml), olanzapine (0.1, 0.5 and 1.0 mg/ml) and aripiprazole (0.05, 0.15 and 0.3 mg/ml) were dissolved on the day of the experiments in the incubation medium (buffer) used for each technique.

Activities of mitochondrial respiratory chain enzymes

In the day of the assays, the samples were frozen and thawed in hypotonic assay buffer three times to fully expose the enzymes to substrates and achieve maximal activities. NADH dehydrogenase (complex I) was evaluated according to the method described by Cassina and Radi (42) by the rate of NADH-dependent ferricyanide reduction at 420 nm. The activities of succinate: 2,6-dichlorophenolindophenol (DCIP) oxidoreductase (complex II) and succinate: cytochrome *c* oxidoreductase (complexes II–III) were determined according to the method of Fischer and colleagues (43). Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at 600 nm. The activity of complexes II–III was measured by cytochrome *c* reduction from succinate. The activity of cytochrome *c* oxidase (complex IV) was assayed according to the method described by Rustin and colleagues (44), measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome *c* at 550 nm. The activities of the mitochondrial respiratory chain complexes were expressed as nmol/min/mg protein.

CK activity

CK activity was measured in brain homogenates pretreated with 0.625 mM lauryl maltoside. Briefly, the reaction mixture consisted of 60 mM Tris–HCl, pH 7.5, containing 7.0 mM phosphocreatine, 9.0 mM MgSO4 and approximately 0.4–1.2 mg protein in a final volume of 100 ml. After 15 min of preincubation at 37 °C, the reaction was then started by the addition of 4.0 mM of ADP and stopped after 10 min by the addition of 20 ml of 50 mM phydroxy-mercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (45). The colour was developed by the addition of 100 ml 2% α-naphtol and 100 ml 0.05% diacetyl in a final volume of 1.0 ml and read spectrophotometrically after 20 min at $\lambda = 540$ nm. Results were expressed as units/min × mg protein.

Statistical analysis

Data were analysed by one-way analysis of variance followed by the Tukey test when F was significant and are expressed as mean ± SD. All analyses were performed using the Statistical Package for the Social Science (SPSS) software (IBM Corporation, NY, USA).

Results

In this study, we evaluated the effects of clozapine, olanzapine and aripiprazole on activities of mitochondrial respiratory chain enzymes and CK activity in some brain areas (hippocampus, striatum, cerebellum, prefrontal cortex and posterior cortex) of rats. It can be seen in Fig. 1a that complex I activity was increased in prefrontal cortex and striatum by olanzapine at 0.5 mg/ml and higher concentrations (prefrontal cortex $p = 0.005$ and $p = 0.001$; striatum $p = 0.004$ and $p = 0.000$). As shown in Fig. 1b, olanzapine at 0.5 and 1.0 mg/ml increased complex II activity in cerebellum, striatum and posterior cortex (cerebellum $p = 0.003$ and $p = 0.000$; striatum $p = 0.002$ and $p = 0.000$; posterior cortex $p = 0.002$ and $p = 0.000$), whereas prefrontal cortex and hippocampus were not affected. Furthermore, olanzapine at 0.5 mg/ml and higher concentrations increased the activity of complexes II–III in prefrontal cortex, striatum and posterior cortex (prefrontal cortex $p = 0.012$ and $p = 0.002$; striatum $p = 0.000$ and $p = 0.000$; posterior cortex $p = 0.000$ and $p = 0.000$). In cerebellum and hippocampus, olanzapine increased the activity of complexes II–III only in

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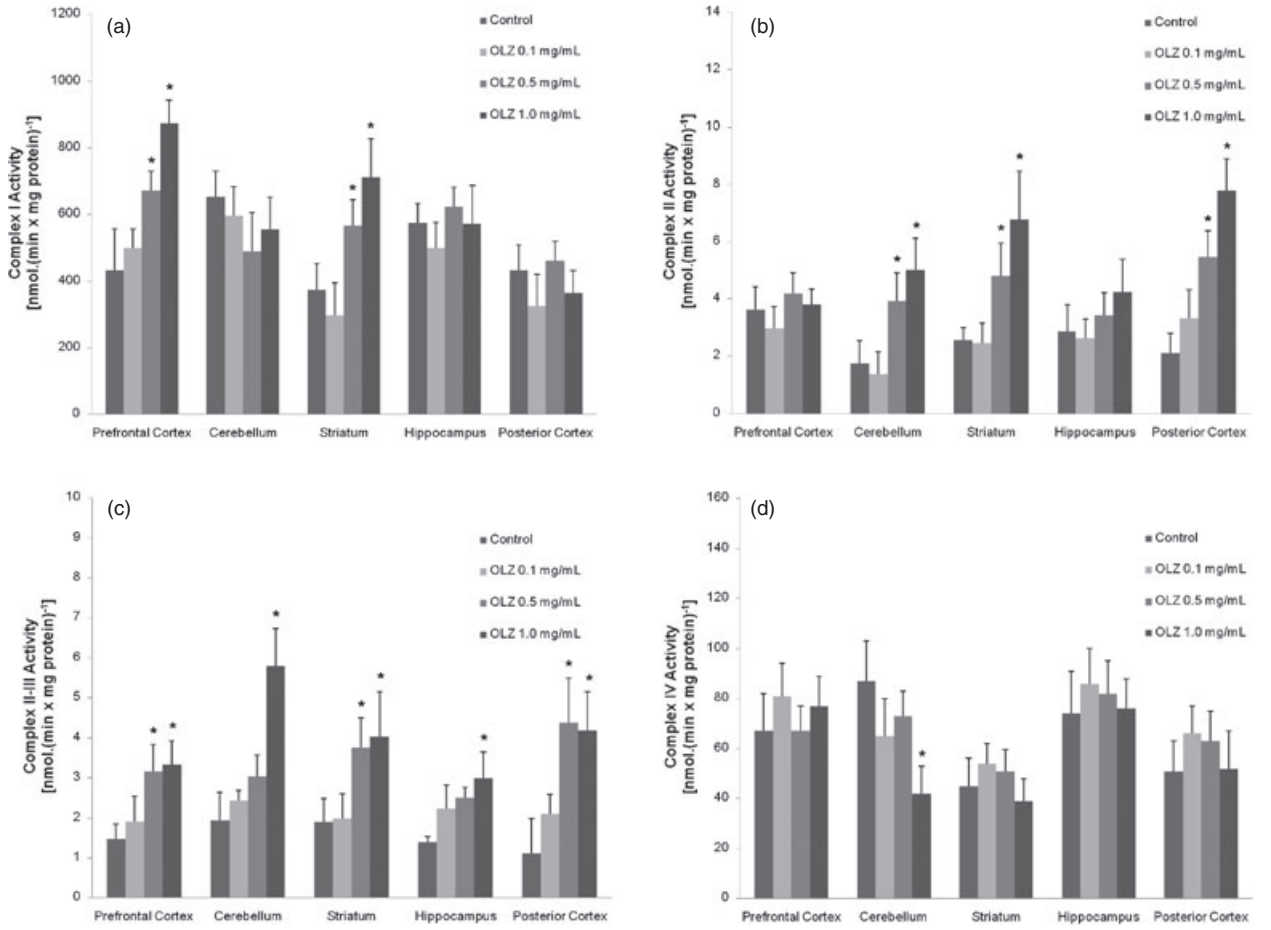

Fig. 1. In vitro effect of olanzapine on the activities of complexes I (a), II (b), II–III (c) and IV (d) in the brain of rats. Data were analysed by Tukey test and are expressed as mean ± SD ($n = 6$). Different from control, *$p < 0.05$.

the higher dose (cerebellum $p = 0.000$; hippocampus $p = 0.000$) (Fig. 1c). On the other hand, olanzapine inhibited complex IV activity in cerebellum only in the higher dose (cerebellum $p = 0.001$), without affecting prefrontal cortex, striatum, hippocampus and posterior cortex (Fig. 1d).

The next set of experiments was performed in order to evaluate the *in vitro* effect of clozapine on the respiratory chain complex activities. Figure 2 shows that clozapine did not affect the activities of complexes I, II and II–III in the cerebral regions evaluated in this study (Fig. 2a–c, respectively). In contrast, clozapine at 0.1 mg/ml and higher concentrations inhibited complex IV activity in prefrontal cortex and hippocampus (prefrontal cortex $p = 0.002$, $p = 0.000$ and $p = 0.004$; hippocampus $p = 0.000$, $p = 0.001$ and $p = 0.000$), on the other hand, as clozapine at 0.5 and 1.0 mg/ml decreased complex IV activity in cerebellum ($p = 0.001$ and $p = 0.006$), but striatum and posterior cortex were not affected (Fig. 2d).

Figure 3 shows that aripiprazole 0.15 and 0.3 mg/ml increased complex I activity in striatum ($p = 0.006$ and $p = 0.003$), but not affect prefrontal cortex, cerebellum, hippocampus and posterior cortex (Fig. 3a). On the other hand, aripiprazole at 0.15 and 0.3 mg/ml increased complex II activity in posterior cortex ($p = 0.002$ and $p = 0.007$). In cerebellum aripiprazole increased complex II activity only in the higher dose ($p = 0.020$). Moreover, prefrontal cortex, striatum and hippocampus were not affected (Fig. 3b). In contrast, the activity of complexes II–III was increased by aripiprazole (0.3 mg/ml) in striatum and hippocampus (striatum $p = 0.038$; hippocampus $p = 0.004$) (Fig. 3c). On the other hand, aripiprazole inhibited complex IV activity in prefrontal cortex only in the higher dose (prefrontal cortex $p = 0.014$), without affecting cerebellum, striatum, hippocampus and posterior cortex (Fig. 3d).

Finally, we tested the influence of olanzapine, clozapine and aripiprazole on the CK activity. It can be seen in Fig. 4a that CK activity was increased by clozapine at 0.5 and 1.0 mg/ml in prefrontal cortex, cerebellum, striatum, hippocampus and posterior cortex of rats (prefrontal cortex $p = 0.000$ and $p = 0.000$; cerebellum $p = 0.015$ and $p = 0.000$;

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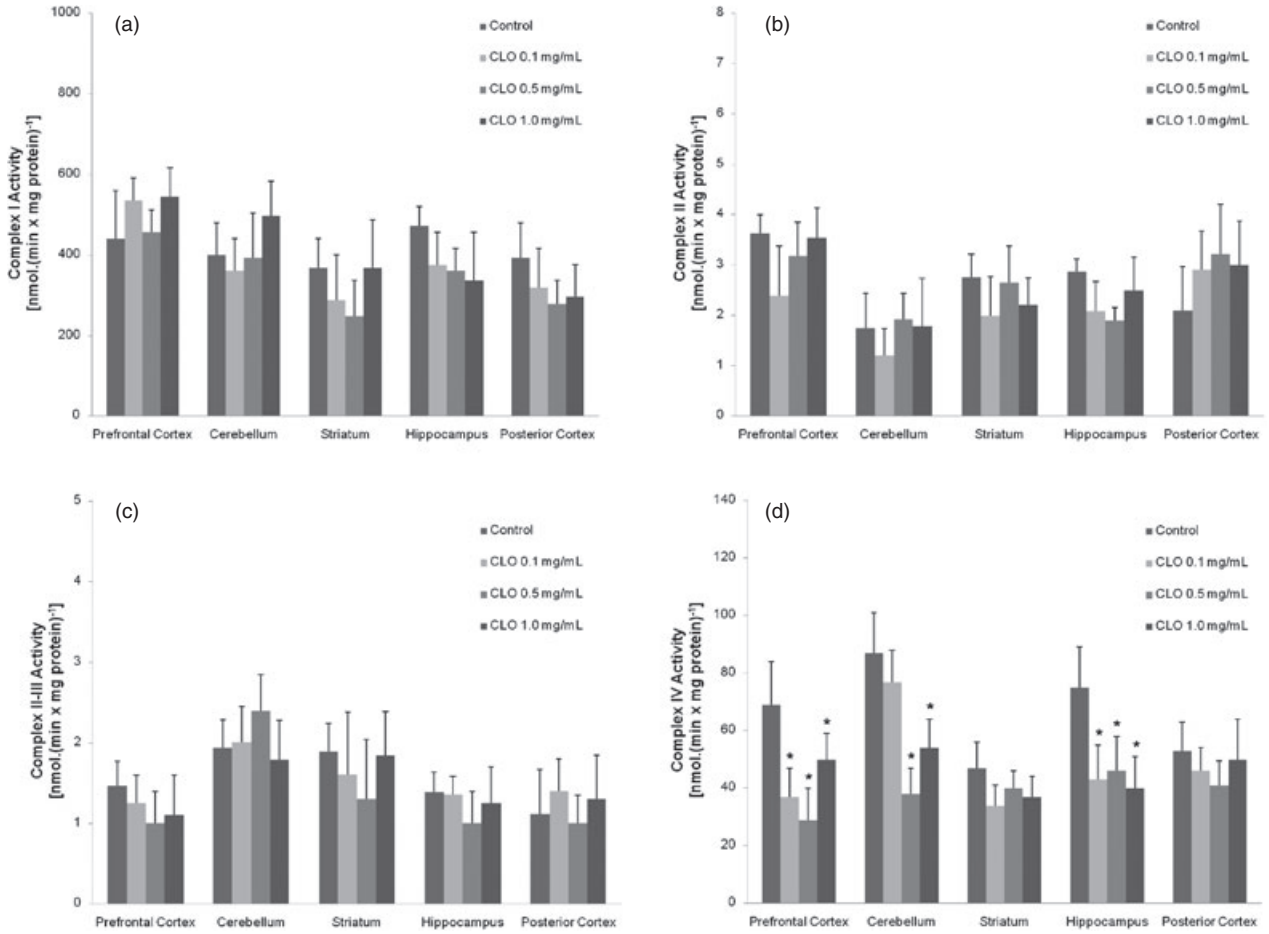

Fig. 2. In vitro effect of clozapine on the activities of complexes I (a), II (b), II–III (c) and IV (d) in the brain of rats. Data were analysed by Tukey test and are expressed as mean ± SD ($n = 6$). Different from control, $^*p < 0.05$.

striatum $p = 0.018$ and $p = 0.000$; hippocampus $p = 0.002$ and $p = 0.009$; posterior cortex $p = 0.001$ and $p = 0.000$). Olanzapine and aripiprazole did not affect CK activity in either tested structure (Fig. 4b and c, respectively).

Discussion

Dysfunction in brain metabolism is related to neuropsychiatric disorders, such as schizophrenia, depression and bipolar disorder (1–3,10). Some evidence also point to the possibility that drugs used in the treatment of such disorders modulate energy metabolism, especially by increasing it. In this context, we reported recently that mood stabilisers prevent and reverse inhibitory effect on cytrate synthase activity caused by amphetamine in an animal model of mania (46). Regarding antipsychotics, reports from literature show that the atypical antipsychotic olanzapine only inhibited succinate dehydrogenase (SDH) in cerebellum and aripiprazole increased the same enzyme in prefrontal cortex, whereas clozapine inhibited the enzyme only in striatum.

Volz and colleagues (47) observed decreased levels of ATP in the frontal lobes of antipsychotic-free patients. Furthermore, reports from literature show that ATP levels may inversely correlate with the degree of negative symptoms in schizophrenic patients (48). Additionally, two groups reported a lower ATP concentration in the basal ganglia of medicated schizophrenic patients, suggesting an imbalance between ATP production and ATP utilisation by oxidative phosphorylation in this disorder (49). Andreassen and colleagues (50) verified that mild mitochondrial impairment in combination with neuroleptics results in striatal excitotoxic neurodegeneration, which may underlie the development of persistent vacuous chewing movements in rats.

It is well described that some side-effects of antipsychotics limited their long-term use and are probably associated to oxidative stress (51,52) and metabolism impairment (53). The biochemical and physiological characteristics of the brain, with high-unsaturated phospholipids content and energy requirement, make this organ particularly susceptible to free radical-mediated damage (20,28). It is

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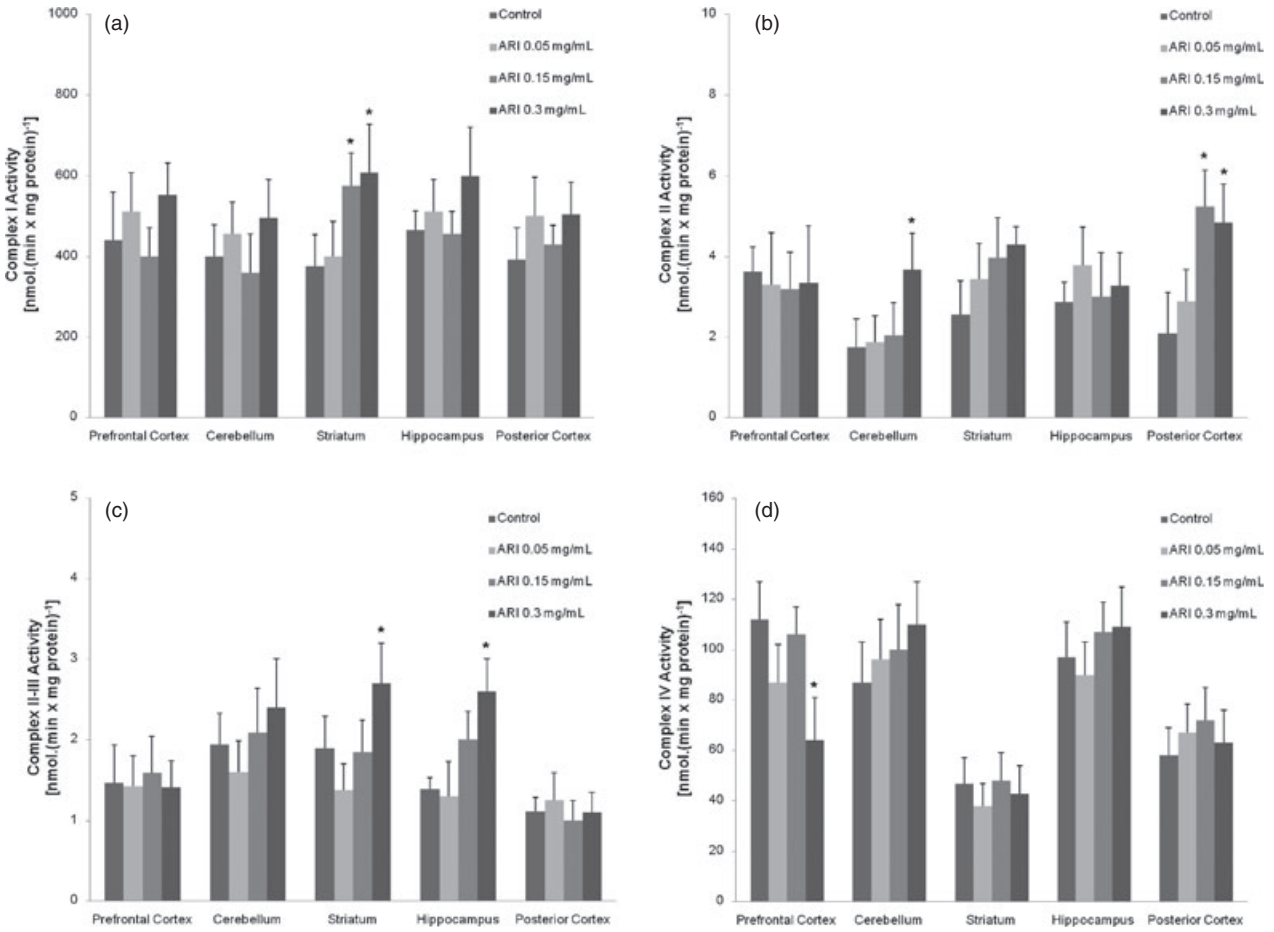

Fig. 3. In vitro effect of aripiprazole on the activities of complexes I (a), II (b), II–III (c) and IV (d) in the brain of rats. Data were analysed by Tukey test and are expressed as mean ± SD ($n = 6$). Different from control, $*p < 0.05$.

well known that mitochondrial oxidative phosphorylation system generates ROS and the electron transport chain, mainly complexes I and III, is vulnerable to damage by them (54,55). In this context, several studies show that antipsychotics cause oxidative stress (17,40). Augmented ROS production causes defects in the mitochondrial genome, leading to impaired oxidative phosphorylation, which not only limits ATP generation but also further promotes ROS production (33). Moreover, oxidative damage can be cause or consequence of mitochondrial dysfunction (20,55).

In this study, we observed that olanzapine and aripiprazole increased the activities of respiratory chain complexes. On the other hand, complex IV activity was inhibited by clozapine, olanzapine and aripiprazole. Prince and colleagues (3) evaluated regional alterations in neuronal functional activity in rat brain using complex IV histochemistry following chronic treatment with haloperidol, fluphenazine and clozapine for 28 days. The authors verified that increases in complex IV activity were evident in the frontal cortex of all treated animals. Moreover, clozapine and fluphenazine, but not haloperidol, caused significant increases in complex IV activity in other areas. The authors also suggest that neuroleptics achieve their therapeutic effects primarily via an enhancement of brain function in the frontal cortex, but also point to other brain regions which may be involved in the actions of these drugs (3). In contrast, ours result suggests that chronic treatment using clozapine increases complex IV activity by an indirect mechanism, probably mediated by signal transduction and/or gene expression pathways.

The creatine/phosphocreatine/CK system is important for normal energy homeostasis by exerting several integrated functions, such as temporary energy buffering, metabolic capacity, energy transfer and metabolic control (8,56). The brain of adult rats, like other tissues with high and variable rates of ATP metabolism, presents high phosphocreatine concentration and CK activity. It has been widely shown that a decrease in CK activity is associated with a neurodegenerative pathway that results in neuronal loss following brain ischaemia (25),

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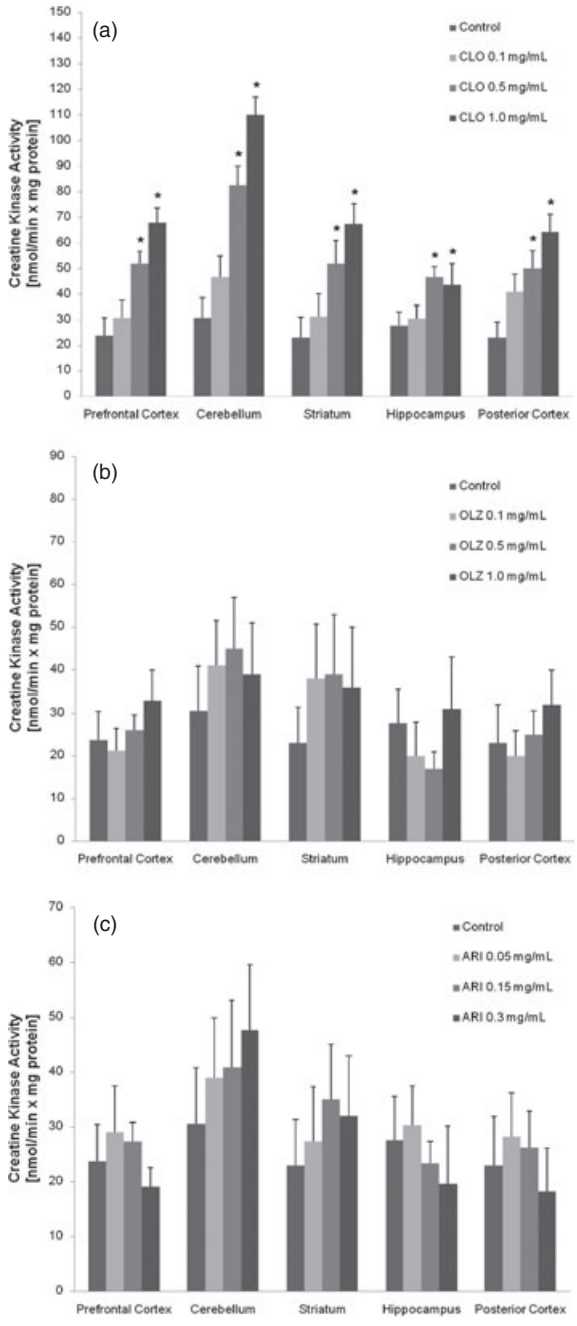

Fig. 4. In vitro effect of olanzapine (a), clozapine (b) and aripiprazole (c) on CK activity in the brain of rats. Data were analysed by Tukey test and are expressed as mean ± SD ($n = 6$). Different from control, $^*p < 0.05$.

neurodegenerative diseases (26,27) and other pathological states (29,30).

Considering that CK is sensitive to free radicals (57) and that we have recently shown that antipsychotics lead to oxidative stress, we speculate that the enzyme inhibition *in vivo* may occur by the oxidation of thiol groups of its structure. In this context, Assis and colleagues (38) observed that chronic administration of clozapine inhibited CK activity in cerebellum and prefrontal cortex. Moreover, the increase *in vitro* CK activity by clozapine differs from *in vivo* studies; thus we suggest that the increased activity occur by direct mechanisms, whereas inhibition *in vivo* may occur by the oxidation of thiol groups of its structure.

Acknowledgements

This research was supported by grants from Programa de Pós-graduação em Ciências da Saúde – Universidade do Extremo Sul Catarinense (UNESC), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Instituto Nacional de Ciência e Tecnologia Translacional em Medicina (INCT).

References

1. Albert KA, Hemmings HC, Adamo AI et al. Evidence for decreased DARPP-32 in the prefrontal cortex of patients with schizophrenia. Arch Gen Psychiatry 2002;**59**:705–712.
2. Konradi C, Eaton M, MacDonald ML, Walsh J, Benes FM, Heckers S. Molecular evidence for mitochondrial dysfunction in bipolar disorder. Arch Gen Psychiatry 2004;**1**:300–308.
3. Prince JA, Yassin MS, Oreland L. A histochemical demonstration of altered cytochrome oxidase activity in the rat brain by neuroleptics. Eur Neuropsychopharmacol 1998;**8**:1–6.
4. Angelucci F, Mathe AA, Aloe L. Neurotrophic factors and CNS disorders: findings in rodent models of depression and schizophrenia. Prog Brain Res 2004;**146**:151–165.
5. Angelucci F, Oliviero A, Pilato F et al. Transcranial magnetic stimulation and BDNF plasma levels in amyotrophic lateral sclerosis. Neuroreport 2004;**15**:717–720.
6. Duman RS. Pathophysiology of depression: the concept of synaptic plasticity. Eur Psychiatry 2002;**17**:306–310.
7. Duman RS. Synaptic plasticity and mood disorders. Mol Psychiatry 2002;**7**:29–34.
8. Schlattner U, Wallimann T. Octamers of mitochondrial creatine kinase isoenzymes differ in stability and membrane binding. J Biol Chem 2000;**275**:17314–17320.
9. Velligan DI, Newcomer J, Pultz J et al. Does cognitive function improve with quetiapine in comparison to haloperidol? Schizophr Res 2002;**53**:239–248.
10. Kato T, Kato N. Mitochondrial dysfunction in bipolar disorder. Bipolar Disord 2000;**2**:180–190.
11. Fatemi SH, Laurence JA, Araghi-Niknam M et al. Glial fibrillary acidic protein is reduced in cerebellum of subjects with major depression, but not schizophrenia. Schizophr Res 2004;**69**:317–323.
12. Machado-Vieira R, Lara DR, Portela LV et al. Elevated serum S100B protein in drug-free bipolar patients during first manic episode: a pilot study. Eur Neuropsychopharmacol 2002;**12**:269–272.
13. Rothermundt M, Missler U, Arolt V et al. Increased S100B blood levels in unmedicated and treated schizophrenic patients are correlated with negative symptomatology. Mol Psychiatry 2001;**6**:445–449.
14. Schroeter ML, Abdul-Khaliq H, Fruhauf S et al. Serum S100B is increased during early treatment with antipsychotics and in deficit schizophrenia. Schizophr Res 2003;**62**:231–236.

https://doi.org/10.1111/j.1601-5215.2012.00650.x Published online by Cambridge University Press

15. Carlson CD, Cavazzoni PA, Berg PH, We HI, Beasley CM, Kane JM. An integrated analysis of acute treatment-emergent extrapyramidal syndrome in patients with schizophrenia during olanzapine clinical trials: comparisons with placebo, haloperidol, risperidone, or clozapine. J Clin Psychiatry 2003;**64**:898–906.
16. Burris KD, Molski TF, Xu C et al. Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D2 receptors. J Pharmacol Exp Ther 2002; **302**:381–389.
17. Jordan S, Koprivica V, Chen R, Tottori K, Kikuchi T, Altar CA. The antipsychotic aripiprazole is a potent, partial agonist at the human 5-HT_{1A} receptor. Eur J Pharmacol 2002;**441**:137–140.
18. Beuzen JN, Taylor N, Wesnes K, Wood A. A comparison of the effects of olanzapine, haloperidol and placebo on cognitive and psychomotor functions in healthy elderly volunteers. J Psychopharmacol 1999;**13**:152–158.
19. Calabrese V, Scapagnini G, Giuffrida-Stella AM, Bates TE, Clark JB. Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. Neurochem Res 2001;**26**: 739–764.
20. Madrigal JL, Olivenza R, Moro MA et al. Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain. Neuropsychopharmacology 2001;**24**:420–429.
21. Fattal O, Budur K, Vaughan AJ, Franco K. Review of the literature on major mental disorders in adult patients with mitochondrial diseases. Psychosomatics 2006;**47**:1–7.
22. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem J 1992;**281**:21–40.
23. Bessman SP, Carpenter CL. The creatine-creatine phosphate energy shuttle. Annu Rev Biochem 1985;**54**:831–865.
24. Tsuji T, Nakamura Y, Ogata T, Shibata T, Kataoka K. Rapid decrease in ATP content without recovery phase duringglutamate-induced cell death in cultured spinal neurons. Brain Res 1994;**62**:289–292.
25. Tomimoto H, Yamamoto K, Homburger HA, Yanagihara T. Immunoelectron microscopic investigation of creatine kinase BB-isoenzyme after cerebral ischemia in gerbils. Acta Neuropathol 1993;**86**:447–455.
26. Aksenov M, Aksenova M, Butterfield DA, Markesbery WR. Oxidative modification of creatine kinase BB in Alzheimer's disease brain. J Neurochem 2000;**74**: 2520–2527.
27. David S, Shoemaker M, Haley BE. Abnormal properties of creatine kinase in alzheimer's disease brain: correlation of reduced enzyme activity and active site photolabeling with aberrant cytosol-membrane partitioning. Mol Brain Res 1998;**54**:276–287.
28. Gross WL, Bak MI, Ingwall JS et al. Nitric oxide inhibits creatine kinase and regulates rat heart contractile reserve. Proc Natl Acad Sci U S A 1996;**93**:5604–5609.
29. Streck EL, Amboni G, Scaini G et al. Brain creatine kinase activity in an animal model of mania. Life Sci 2008;**82**:424–429.
30. Lee JM, Grabb MC, Zipfel GJ, Choi DW. Brain tissue responses to ischemia. J Clin Invest 2000;**106**:723–731.
31. Blass JP. Brain metabolism and brain disease: is metabolic deficiency the proximate cause of Alzheimer dementia? J Neurosci Res 2001;**66**:851–856.
32. Brennan WA, Bird ED, Aprille JR. Regional mitochondrial respiratory activity in Huntington's disease brain. J Neurochem 1985;**44**:1948–1950.
33. Gruno M, Peet N, Tein A et al. Atrophic gastritis: deficient complex I of the respiratory chain in the mitochondria of corpus mucosal cells. J Gastroenterol 2008;**43**:780–788.
34. Schurr A. Energy metabolism, stress hormones and neural recovery from cerebral ischemia/hypoxia. Neurochem Int 2002;**41**:1–8.
35. Prabakaran S, Swatton JE, Ryan MM et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. Mol Psychiatry 2004;**9**:684–697.
36. Rothermundt M, Ponath G, Arolt V. S100B in schizophrenic psychosis. Int Rev Neurobiol 2004;**59**:445–470.
37. Weis S, Llenos IC. GFAP-immunopositive astrocytes in schizophrenia. Schizophr Res 2004;**67**:293–295.
38. Assis LC, Scaini G, Di-Pietro PB et al. Effect of antipsychotics on creatine kinase activity in rat brain. Basic Clin Pharmacol Toxicol 2007;**101**:315–319.
39. Streck EL, Rezin GT, Barbosa LM, Assis LC, Grandi E, Quevedo J. Effect of antipsychotics on succinate dehydrogenase and cytochrome oxidase activities in rat brain. Naunyn Schmiedebergs Arch Pharmacol 2007;**376**: 127–133.
40. Polydoro M, Schröder N, Lima MN et al. Haloperidol- and clozapine-induced oxidative stress in the rat brain. Pharmacol Biochem Behav 2004;**78**:751–756.
41. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;**193**:265–267.
42. Cassina A, Radi R. Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. Arch Biochem Biophys 1996;**328**:309–316.
43. Fischer JC, Ruitenbeek W, Berden JA et al. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. Clin Chim Acta 1985;**153**:23–36.
44. Rustin P, Chretien D, Bourgeron T et al. Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta 1994;**228**:35–51.
45. Hughes BP. A method for estimation of serum creatine kinase and its use in comparing creatine kinase and aldolase activity in normal and pathologic sera. Clin Chim Acta 1962;**7**:597–604.
46. Corrêa C, Amboni G, Assis LC et al. Effects of lithium and valproate on hippocampus citrate synthase activity in an animal model of mania. Prog Neuropsychopharmacol Biol Psychiatry 2007;**31**:887–891.
47. Volz HR, Riehemann S, Maurer I et al. Reduced phosphodiesters and high-energy phosphates in the frontal lobe of schizophrenic patients: A (31)P chemical shift spectroscopic-imaging study. Biol Psychiatry 2000;**47**: 954–961.
48. Volz HP, Rzanny R, Rossger G et al. Decreased energy demanding processes in the frontal lobes of schizophrenics due to neuroleptics? A 31Pmagneto-resonance spectroscopic study. Psychiatry Res 1997;**76**:123–129.
49. Deicken RF, Calabrese G, Merrin EL, Fein G, Weiner MW. Basal ganglia phosphorous metabolism in chronic schizophrenia. Am J Psychiatry 1995;**152**:126–129.

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50. Andreassen OA, Ferrante RJ, Beal MF, Jorgensen HA. Oral Dyskinesias and striatal lesions in rats after long-term co-treatment with haloperidol and 3-nitropropionic acid. Neuroscience 1998;**87**:639–648.
51. Cadet JL, Lohr JB. Possible involvement of free radicals in neuroleptic-induced movement disorders. Evidence from treatment of tardive dyskinesia with vitamin E. Ann N Y Acad Sci 1989;**570**:176–185.
52. Lohr JB, Cadet JL, Lohr MA et al. Vitamin E in the treatment of tardive dyskinesia: the possible involvement of free radical mechanisms. Schizophr Bull 1988;**14**:291–296.
53. Arnaiz SL, Coronel MF, Boveris A. Nitric oxide, superoxide and hydrogen peroxide production in brain mitochondria after haloperidol treatment. Nitric Oxide 1999;**3**:235–243.
54. Adam-Vizi V. Production of reactive oxygen species in brain mitochondria: contribution by electron transport chain and non-electron transport chain sources. Antioxid Redox Signal 2005;**7**:1140–1149.
55. Torres RL, Torres ILS, Gamaro GD et al. Lipid peroxidation and total radical-trapping potential of the lungs of rats submitted to chronic and sub-chronic stress. Braz J Med Biol Res 2004;**37**:185–192.
56. Khuchua ZA, Qin W, Boero J et al. Octamer formation and coupling of cardiac sarcomeric mitochondrial creatine kinase are mediated by charged N-terminal residues. J Biol Chem 1998;**273**:22990–22996.
57. Wolosker H, Panizzutti R, Englender S. Inhibition of creatine kinase by S-nitrosoglutathione. FEBS Lett 1996;**392**:274–276.

https://doi.org/10.1111/j.1601-5215.2012.00650.x Published online by Cambridge University Press