Acta Neuropsychiatrica

Acta Neuropsychiatrica 2011: 23: 282–291
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DOI: 10.1111/j.1601-5215.2011.00569.x

ACTA NEUROPSYCHIATRICA

Olanzapine plus fluoxetine treatment alters mitochondrial respiratory chain activity in the rat brain

Agostinho FR, Réus GZ, Stringari RB, Ribeiro KF, Ferreira GK, Jeremias IC, Scaini G, Rezin GT, Streck EL, Quevedo J. Olanzapine plus fluoxetine treatment alters mitochondrial respiratory chain activity in the rat brain.

Background: Evidence is emerging for the role of dysfunctional mitochondria in pathophysiology and treatment of mood disorders. In this study, we evaluated the effects of acute and chronic administration of fluoxetine (FLX), olanzapine (OLZ) and the combination of FLX/OLZ on mitochondrial respiratory chain activity in the rat brain.
Methods: For acute treatment, Wistar rats received one single injection of OLZ (3 or 6 mg/kg) and/or FLX (12 or 25 mg/kg) and for chronic treatment, rats received daily injections of OLZ (3 or 6 mg/kg) and/or FLX (12 or 25 mg/kg) for 28 days and we evaluated the activity of mitochondrial respiratory chain complexes I, II, II–III and IV in prefrontal cortex, hippocampus and striatum.
Results: Our results showed that both acute and chronic treatments with FLX and OLZ alone or in combination altered respiratory chain complexes activity in the rat brain, but in combination we observed larger alterations.
Conclusions: Finally, these findings further support the hypothesis that metabolism energy could be involved in the treatment with antipsychotics and antidepressants in combination to mood disorders.

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Keywords: bipolar depression; bipolar disorder; fluoxetine; mitochondrial respiratory chain; olanzapine

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Significant outcomes

- Effects of FLX and OLZ alone or in combination on mitochondrial respiratory chain.
- Acute and chronic treatment of FLX and OLZ alone or in combination altered respiratory chain complexes activity.
- In combination, FLX and OLZ caused larger alterations.

Limitations

- The results did not follow a pattern. Sometimes FLX and/or OLZ increased and sometimes decreased the activity of the complexes.

Introduction

Mood disorders are among the most prevalent forms of mental illness. Severe forms of depression affect 2–5% of the US population, and up to 20% suffer from milder forms of the illness. Another roughly 1–2% are afflicted by bipolar disorder (BD) or its less severe variants (1,2) and are associated with higher rates of suicide and work loss (3–5).

Tissues with high energy demands, such as the brain, contain a large number of mitochondria and are therefore more susceptible to reduction of the aerobic metabolism (6) Mitochondrial disease results from a malfunction in biochemical cascade and the damage to the mitochondrial electron transport chain has been suggested to be an important factor in the pathogenesis of a range of neuropsychiatries disorders, such as BD, depression and schizophrenia (7,8). Several studies have shown that the abnormalities in energy metabolism lead to cellular degeneration (9). This effect may occur because when the mitochondrial dysfunction is severe it can lead to cell death by apoptosis or necrosis (10,11). In fact, mitochondria are involved in essential processes, such as apoptosis and calcium homeostasis (12–14), which are involved in cell death.

Mitochondria are intracellular organelles that play a crucial role in ATP production (9). Most cell energy is obtained through oxidative phosphorylation, a process requiring the action of various respiratory enzyme complexes located in a special structure of the inner mitochondrial membrane, the mitochondrial respiratory chain (15). In most organisms, the mitochondrial respiratory chain is composed of four complexes, where the electron transport couples with translocation of protons from the mitochondrial matrix to the intermembrane space. The generated proton gradient is used by ATP synthase to catalyse the formation of ATP by the phosphorylation of ADP (7,16).

A fixed combination of antipsychotic and antidepressant drugs was widely used in medicine and, at one time, was common in psychiatry. A generation ago, combinations of antidepressants with either antipsychotics [e.g. amitriptyline and perphenazine (Etrafon™; Schering–Bayer HealthCare Pharmaceuticals, Berlin, Germany and Triavil™; Merck & Company Inc., Whitehouse Station, NJ, USA)] or benzodiazepines [e.g. amitriptyline and chlordiazepoxide (Limbitrol™; Valeant Pharmaceuticals International, USA/Valeant Farmacêutica do Brasil, SP, Brazil)] were widely used by both psychiatrists and other medical practitioners (17). Recently, a fixed combination of the antipsychotic drug olanzapine (OLZ) and the antidepressant fluoxetine (FLX) (Symbyax™; Eli Lilly and Company, Indianapolis, IN, USA) has been introduced for the treatment of BD (3,18). In a controlled study by Shelton et al. (19), subjects with treatment-resistant depression received OLZ alone, FLX alone or a combination of both; the combination was associated with significantly greater and faster improvement than was either drug alone. Although there is a clear clinical benefit from this combination, the precise neural mechanisms responsible for its efficacy are not clearly understood. Therefore, it is important to investigate the mechanisms of action of this combination in order to not only better understand the aetiology of the clinical syndromes, but also to eventually facilitate the development of improved drugs to treat them (4,20).

Considering the effects of OLZ, FLX and these combinations on brain energy metabolism are still unknown, we evaluated the effects of these drugs on mitochondrial respiratory chain in the rat prefrontal cortex, hippocampus and striatum. It is important to note that we chose the prefrontal cortex, hippocampus and striatum in this study because these brain areas are implicated in mood disorders (21,22).

Material and methods

Animals

Male adult Wistar rats (60 days old) were obtained from UNESC (Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil) breeding colony. They were housed five per cage with food and water available *ad libitum* and were maintained on a 12-h light/dark cycle (lights on at 7:00 h). All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care and with approval by local Ethics Committee under protocol number 510/2006.

Drugs and treatments

OLZ (Zyprexa™) and FLX (Prozac™) were provided from Eli Lilly do Brasil Ltda, São Paulo, Brazil. Animals received daily intraperitoneal injections of OLZ (3 or 6 mg/kg), FLX (12 or 25 mg/kg) or combination of both drugs for 28 days in two protocols of the chronic model (A and B) and in the acute model for 1 day. Protocols and doses of drugs were performed in accordance with previous studies (23,24). All the drugs were dissolved in saline (0.9% NaCl) solution (vehicle). Control animals received saline (0.9% NaCl; 1.0 ml/kg). In the acute protocol, after the single injection, the animals were killed 2 h later by decapitation, and the prefrontal cortex, hippocampus and striatum were immediately removed. Similarly, in the chronic protocols, the animals were killed 2

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(A) and 24 (B) h after the last injection, and the same areas were removed. The analysis was performed at different times of decapitation (2 and 24 h) after the last injection to be sure that the effects of the studied parameters were because of a chronic effect (23,24). After that, the activity of mitochondrial respiratory chain was measured ($n = 5$ each).

Tissue and homogenate preparation

Hippocampus, striatum and prefrontal cortex were homogenised (1:10, w/v) in SETH (sucrose, EDTA, tris and heparin) buffer at pH 7.4 (250 mM sucrose, 2 mM ethylenediaminetetraacetic acid, 10 mM Trizma base and 50 IU/ml heparin). The homogenates were centrifuged at 800*g* for 10 min and the supernatants kept at −70 °C until used for mitochondrial respiratory chain activity determination. The maximal period between homogenate preparation and enzyme analysis was always <5 days. Protein content was determined by the method described by Lowry et al. (25) using bovine serum albumin as standard.

Respiratory chain enzyme activities

NADH dehydrogenase (complex I) was evaluated by the method described by Cassina and Radi (26) with the rate of NADH-dependent ferricyanide reduction at 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. (27). Complex II activity was measured by following the decrease in absorbance due to the reduction in 2,6-DCIP at 600 nm. Complex II–III activity was measured by cytochrome *c* reduction from succinate at 550 nm. The activity of cytochrome *c* oxidase (complex IV) was assayed according to the method described by Rustin et al. (28) and 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 calculated as nmol/min mg protein.

Statistical analysis

All data are presented as mean ± SEM (standard error of the mean). Differences among experimental groups in the assessment of mitochondrial respiratory chain activity were determined by one-way ANOVA, followed by Tukey *post hoc* test when ANOVA was significant; $p < 0.05$ was considered to be statistically significant.

Results

As depicted in Fig. 1a, complex I activity increased in the prefrontal cortex of rats treated acutely with OLZ 6 mg/kg and OLZ 3 mg/kg plus FLX 12 mg/kg (Fig. 1a; $F = 35.63$; $p < 0.05$); in the hippocampus complex I activity increased with FLX 25 mg/kg (Fig. 1a; $F = 180.27$; $p < 0.05$); in the striatum complex I activity increased with OLZ 6 mg/kg (Fig. 1a; $F = 3.69$; $p < 0.05$). The complex II activity increased in prefrontal cortex (Fig. 1b; $F = 2.28$; $p < 0.05$) and hippocampus (Fig. 1b; $F = 36.43$; $p < 0.05$) after acute treatment with OLZ 6 mg/kg alone. The complex II–III activity increased in the prefrontal cortex (Fig. 1c; $F = 17.03$; $p < 0.05$) with OLZ 3 mg/kg plus FLX 25 mg/kg and OLZ 6 mg/kg plus FLX 25 mg/kg, in the hippocampus (Fig. 1c; $F = 6.92$; $p < 0.05$) with OLZ 3 mg/kg plus FLX 25 mg/kg and OLZ 6 mg/kg plus FLX 25 mg/kg and in the striatum (Fig. 1c; $F = 15.71$; $p < 0.05$) with OLZ 3 mg/kg plus FLX 12 or 25 mg/kg and OLZ 6 mg/kg plus FLX 25 mg/kg. After acute treatment, the complex IV activity increased in the prefrontal cortex (Fig. 1d; $F = 5.7$; $p < 0.05$) with OLZ 3 mg/kg plus FLX 25 mg/kg and OLZ 6 mg/kg plus FLX 12 or 25 mg/kg and in the striatum (Fig. 1d; $F = 8.68$; $p < 0.05$) with OLZ 3 mg/kg plus FLX 12 or 25 mg/kg. In the chronic treatment, when the animals were killed 2 h after the last injection (Fig. 2), there was an increase in complex I activity in the striatum after OLZ 6 mg/kg and FLX 25 mg/kg in combination (Fig. 2a; $F = 4.02$; $p < 0.05$). The complex II activity decreased in the striatum after chronic treatment with OLZ 6 mg/kg plus FLX 12 mg/kg (Fig. 2b; $F = 1.87$; $p < 0.05$) and it was not altered in the prefrontal cortex (Fig. 2b; $F = 0.92$; $p > 0.05$) and hippocampus (Fig. 2b; $F = 1.93$; $p > 0.05$). The complex II–III activity increased in the striatum with OLZ 3 mg/kg alone compared to the control group (Fig. 2c; $F = 7.81$; $p < 0.05$) and it was not altered in the prefrontal cortex (Fig. 2c; $F = 1.87$; $p > 0.05$) and hippocampus (Fig. 2c; $F = 1.91$; $p > 0.05$). The complex IV activity did not alter in the prefrontal cortex (Fig. 2d; $F = 0.36$; $p > 0.05$) and striatum (Fig. 2d; $F = 3.52$; $p < 0.05$) compared to the control group; in contrast, in the hippocampus, the complex IV activity increased after treatment with OLZ 6 mg/kg and FLX 25 mg/kg in combination compared to the control group (Fig. 2d; $F = 2.4$; $p < 0.05$).

In the chronic treatment, when the animals were killed 24 h after the last injection (Fig. 3), we showed that complex I activity decreased in the prefrontal cortex with FLX 12 mg/kg alone compared to the control group (Fig. 3a; $F = 3.9$; $p < 0.05$);

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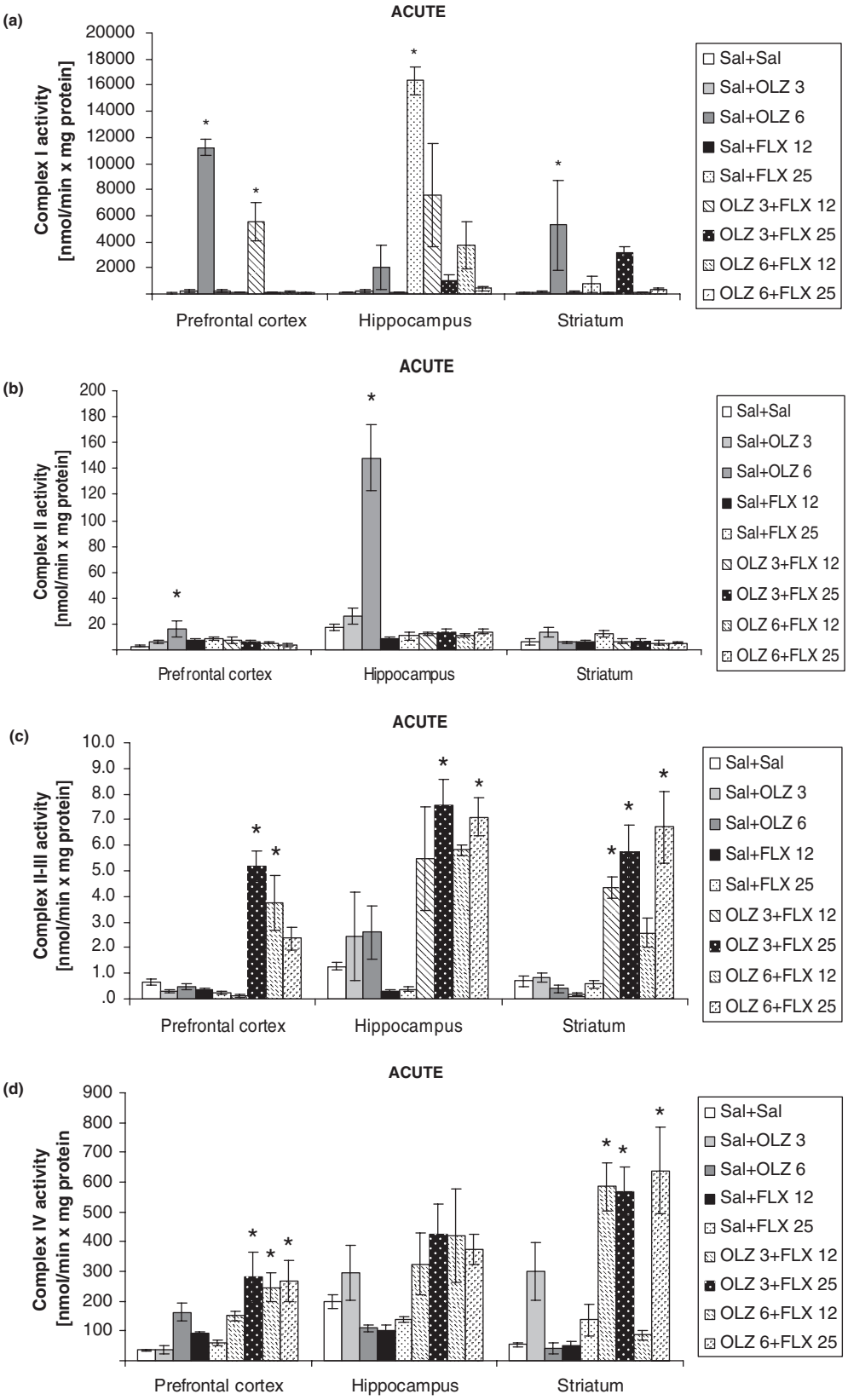

Fig. 1. Effects of the acute administration of olanzapine and fluoxetine on the complex I (a), II (b), II–III (c) and IV (d) activities in the rat prefrontal cortex, hippocampus and striatum. Bars represent means ± SEM (standard error of the mean). $^{*}p < 0.05$ versus saline according to ANOVA followed by Tukey *post hoc* test.

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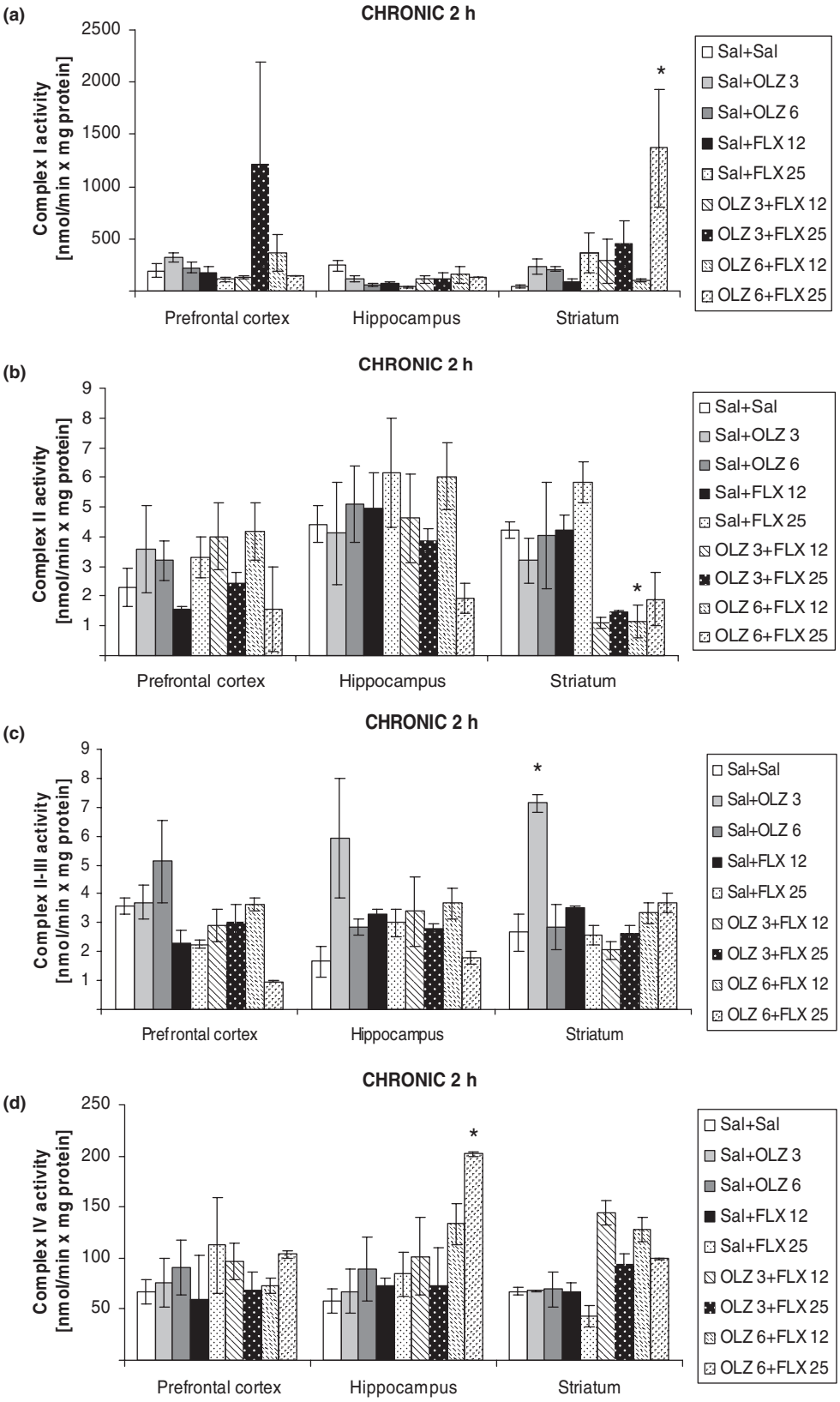

Fig. 2. Effects of the chronic administration of olanzapine and fluoxetine on the complex I (a), II (b), II–III (c) and IV (d) activities in the rat prefrontal cortex, hippocampus and striatum. The animals were killed 2 h after the last administration of the drugs. Bars represent means ± SEM (standard error of the mean). $^{*}p < 0.05$ versus saline according to ANOVA followed by Tukey *post hoc* test.

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however, the complex I activity did not alter in hippocampus (Fig. 3a; $F = 2.21$; $p > 0.05$) and striatum (Fig. 3a; $F = 1.15$; $p > 0.05$). The complex II activity (Fig. 2b) did not alter in the prefrontal cortex ($F = 3.45$), hippocampus ($F = 4.83$) and striatum ($F = 1.39$) compared to the control group. Treatment with FLX 25 mg/kg alone decreased complex II–III activity in the striatum compared to the control group (Fig. 3c; $F = 4.99$; $p < 0.05$). In the hippocampus (Fig. 3c; $F = 3.42$; $p > 0.05$) and prefrontal cortex (Fig. 3c; $F = 2.18$; $p > 0.05$), we did not observe alteration in the complex II–III activity. Figure 3d shows that complex IV activity did not alter in the prefrontal cortex ($F = 2.13$). In the striatum, the complex IV activity increased after treatment with OLZ 6 mg/kg and FLX 12 mg/kg in combination compared to the control group ($F = 8.18$; $p < 0.05$). In contrast, in the hippocampus the complex IV activity decreased after treatment with OLZ 3 and 6 mg/kg alone, as with FLX 12 and 25 mg/kg alone. In addition, OLZ 3 mg/kg plus FLX 12 or 25 mg/kg also decreased the complex IV activity compared to the control group ($F = 6.87$; $p < 0.05$).

Discussion

In this study, we evaluated the effects of the antipsychotic OLZ and the antidepressant FLX (alone or in combination) on mitochondrial respiratory chain activity in the rat brain. We showed that both acute and chronic treatments with FLX and OLZ alone or in combination altered respiratory chain complex activity in the rat brain, but in combination we observed larger alterations. We showed that these alterations were related to treatment regime, complex, brain area and drug concentration.

Recent studies from our group showed that acute administration of FLX inhibited creatine kinase in the rat brain. This study also showed that chronic treatment, when the animals were killed 2 h after the last injection, showed a decrease in the creatine kinase activity after FLX administration, alone or in combination with OLZ. In contrast, when the animals were killed 24 h after the last injection we did not observe alterations in the enzyme (23). In addition, acute, but not chronic treatment with FLX and OLZ alone or in combination increased citrate synthase activity in the rat brain (24). Creatine kinase works as a buffering system of cellular ATP levels and citrate synthase has been used as a quantitative enzyme marker for the presence of intact mitochondria (29). Both enzymes play an important role in brain energy metabolism. In fact, several studies have been appointed to mitochondrial abnormalities in a number of disorders, including depression, BD and schizophrenia (7,30,31).

Studies have identified that some brain regions from BD patients presented a decreased energy metabolism and abnormalities in mitochondrial DNA (32,33). Moreover, reductions of mitochondrial respiratory chain were found in patients with depression, schizophrenia and BD (30,34). Additionally, animal models evaluating the molecular pharmacology of mood stabilising drugs have implicated mitochondrial energy metabolism as a target for these drugs (35,36).

Dror et al. (37) showed alteration in complex I activity and in levels of mRNA and protein of the 24- and 51-kDa iron–sulphur flavoprotein subunits of the complex from platelets of schizophrenia patients, suggesting that these alterations may result in abnormal neural transmission, synaptic plasticity and connectivity, leading to abnormal behavioural symptoms in schizophrenia. Moreover, another study has shown abnormalities in energy metabolism in the basal ganglia of chronic schizophrenics (38). In addition, Iwamoto et al. (32) showed mitochondrial dysfunction in postmortem brains of schizophrenic patients; however, this dysfunction was due to the patients' medication, especially antipsychotics. Additionally, a study showed that OLZ, clozapine and haloperidol inhibited succinate dehydrogenase (an important enzyme of the Krebs cycle and part of the mitochondrial respiratory chain as an electron-transferring protein); however, aripiprazole antipsychotic increased the enzyme in the rat brain (39). Several studies have shown that antipsychotic drugs inhibited the respiratory electron transport chain (40–43). In this study, we showed that OLZ alone or in combination with FLX inhibited the complex IV activity in the hippocampus when the animals were killed 24 h after the last injection, and OLZ in combination with FLX inhibited the complex II activity in the striatum when the animals were killed 2 h after the last injection; however, in most cases, OLZ alone or in combination acted to increase the complex respiratory chain in the rat brain.

The effects of OLZ and FLX found in this study could be also related to oxidative stress. In fact, mitochondria can produce an excess of reactive oxygen species (ROS), which will cause oxidative damage to cellular constituents such as membrane lipids and proteins (44). In addition, mtDNA mutations in elevated production of ROS in turn proved to increase the number of mtDNA mutations (45). Several studies have generally suggested a compromised oxidative stress in psychiatric disorders such as BD, depression and schizophrenia (46–48). Additionally, chronic exposure to antipsychotics, haloperidol and

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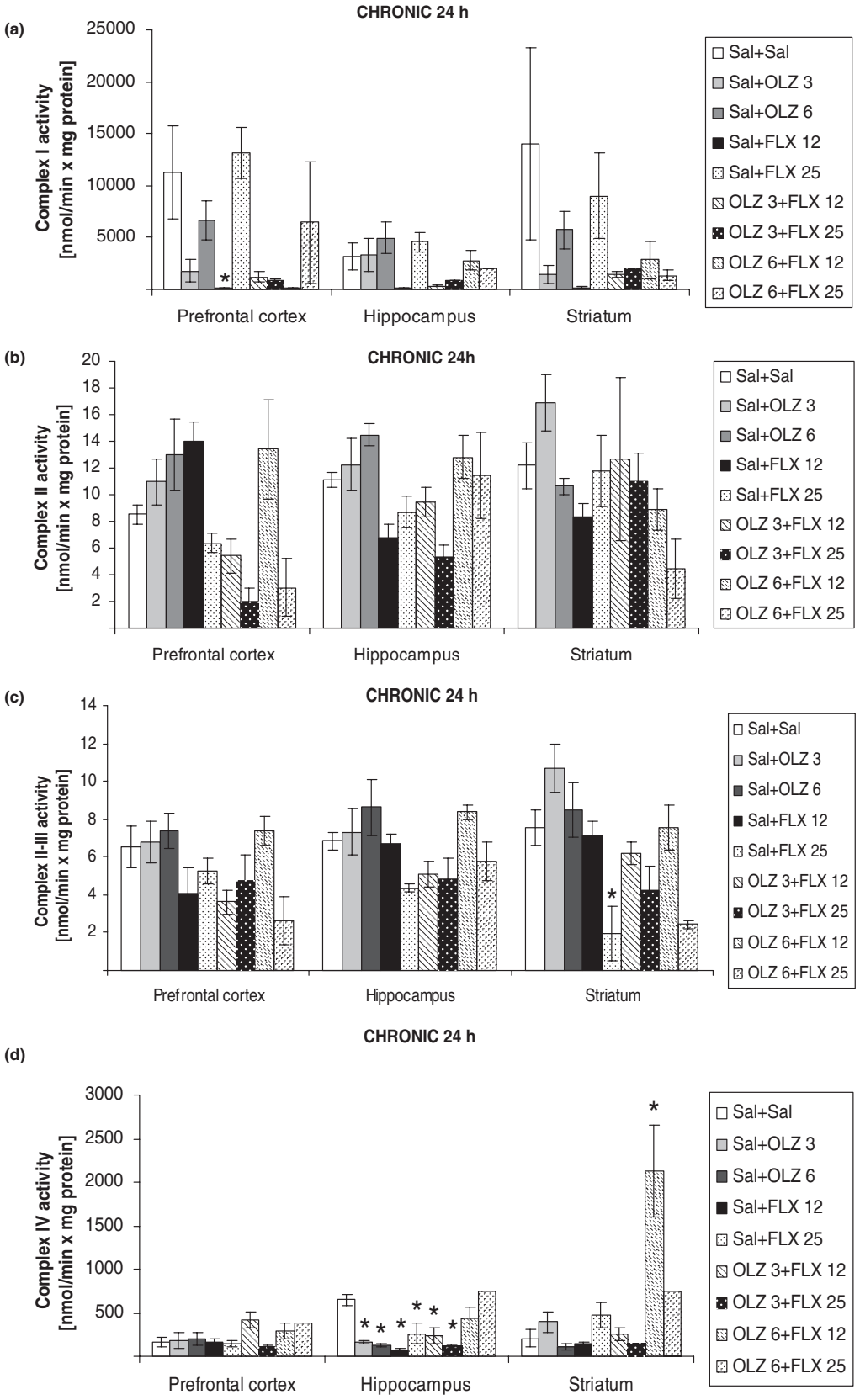

Fig. 3. Effects of the chronic administration of olanzapine and fluoxetine on the complex I (a), II (b), II-III (c) and IV (d) activities in the rat prefrontal cortex, hippocampus and striatum. The animals were killed 24 h after the last administration of the drugs. Bars represent means ± SEM (standard error of the mean). $^{*}p < 0.05$ versus saline according to ANOVA followed by Tukey *post hoc* test.

https://doi.org/10.1111/j.1601-5215.2011.00569.x Published online by Cambridge University Press

clozapine, but not OLZ, caused changes in the activities of antioxidant enzymes and oxidative damage in the rat brain (49,50). Researchers have reported that some side effects of antipsychotics are associated with oxidative stress (51,52) and metabolism impairment (53). Recently, a study from our group showed that OLZ and FLX treatment inhibited creatine kinase activity (23), suggesting that inhibition of enzyme may be associated with the occurrence of some side effects of OLZ and FLX. However, OLZ exerted antioxidant effects through modulating ROS levels, superoxide dismutase activity and Bax expression to provide protective effects against *N*-methyl-4-phenylpyridinium-induced oxidative stress in PC12 cells (54). FLX has also shown an antioxidant effect (55–57).

Reductions in mRNA and proteins of complex I subunits NADH dehydrogenase ubiquinone flavoprotein (*NDUFV1*), NADH–ubiquinone oxidoreductase flavoprotein gene (*NDUFV2*) and NADH dehydrogenase (ubiquinone) Fe-S protein 1(*NDUFS1*) have been shown in the cerebellum postmortem from patients with depression (30). Many animal models of mania and depression have revealed alterations in metabolism energy. Studies from our group showed reduced creatine kinase and citrate synthase activity in brain of rats submitted to the animal model of mania (35,58) Moreover, in another study from our group, it was shown that antidepressants imipramine (59) and paroxetine (60) increased creatine kinase activity in the rat brain, suggesting that the modulation of energy metabolism by antidepressants could be an important mechanism of action of these drugs. Nevertheless, our group also showed that mitochondrial respiratory chain complexes I, II–III and IV were inhibited after chronic mild stress in the cerebral cortex and cerebellum (61). Madrigal et al. (16) also reported that complexes I–III and II–III of mitochondrial respiratory chain were inhibited in rat brains after chronic stress (immobilisation for 6 h during 21 days). Hroudova and Fisar (62) showed that several antidepressant drugs inhibited complexes I and IV of the mitochondrial respiratory chain, suggesting that in pathophysiology of mood disorders therapeutic effects of antidepressant could have changes in energetic metabolism of cells determined by mitochondria.

In clinical practice, atypical antipsychotic drugs in combination with antidepressant drugs have been used as a strategy to treat (63) treatment-resistant depression (1,64,65) and psychotic depression (66). In an elegant controlled study, Matthews et al. (67) showed that subjects with treatment-resistant depression received OLZ and FLX alone or a combination of both; the combination was associated with significantly greater and faster improvement than was either drug alone. In this study, we also showed greater effects of OLZ and FLX in combination under metabolism energy parameters, antidepressant FLX and antipsychotic OLZ alone or in combination increased or decreased mitochondrial respiratory chain, dependent on treatment regime, enzymatic complex, brain area and drug concentration. The reason for this different alteration in this study is unclear, but could be related to desensitisation of the effects of repeated OLZ and FLX administration or to the adaptation mechanism of mitochondria. The differences of OLZ and FLX found in these findings could be related to brain distribution of the drugs or differences in the toxicity of its metabolites.

In conclusion, taking together the present findings and evidence from the literature, we hypothesise that FLX and OLZ in combination could be involved in mitochondrial function, which is altered in several mood disorders. However, it remains to be seen if effects of the combination of drugs on the mitochondrial respiratory chain are related to the therapeutic or side effects of pharmacotherapy.

Acknowledgements

This study was supported in part by grants from 'Conselho Nacional de Desenvolvimento Científico e Tecnológico' (CNPq-Brazil – JQ, ELS), the Instituto Cérebro e Mente (JQ) and UNESC (JQ and ELS). JQ and ELS are recipients of CNPq (Brazil) Productivity fellowships. GZR is holder of an FAPESC studentship.

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