Acta Neuropsychiatrica
An International Journal of Translational Neuropsychiatry

Acta Neuropsychiatrica 2012: 24: 147–154

DOI: 10.1111/j.1601-5215.2011.00592.x

ACTA NEUROPSYCHIATRICA

Differential effects of escitalopram administration on metabolic parameters of cortical and subcortical brain regions of Wistar rats

Gonçalves CL, Rezin GT, Ferreira GK, Jeremias IC, Cardoso MR, Carvalho-Silva M, Zugno AI, Quevedo J, Streck EL. Differential effects of escitalopram administration on metabolic parameters of cortical and subcortical brain regions of Wistar rats.

Objective: Considering that mitochondria may be drug targets and some characteristics of drug–mitochondria interactions may still be misjudged because of the difficulty in foreseeing and understanding all possible implications of the complex pathophysiology of mitochondria, our study aimed to investigate the effect of escitalopram on the activity of enzymes of mitochondrial energy metabolism.
Methods: Animals received daily administration of escitalopram dissolved in saline [10 mg/kg, intraperitoneal (IP)] at 1.0 ml/kg volume for 14 days. Control rats received an equivalent volume of saline, 1.0 ml/kg (IP), for the same treatment period. Twelve hours after last injection, rats were killed by decapitation and brain areas were rapidly isolated. The samples were homogenised and the activities of mitochondrial respiratory chain complexes, some enzymes of Krebs cycle (citrate synthase, malate dehydrogenase and succinate dehydrogenase) and creatine kinase were measured.
Results: We verified that chronic administration of escitalopram decreased the activities of complexes I and II–III in cerebellum, hippocampus, striatum and posterior cortex whereas prefrontal cortex was not affected. Complex II activity was decreased only in striatum without affecting prefrontal cortex, hippocampus, cerebellum and posterior cortex. However, chronic administration of escitalopram did not affect complex IV and enzymes of Krebs cycle activities as well as creatine kinase.
Conclusion: In this study we showed a decrease in the activities of complexes I and II–III in most of the brain structures analysed and complex II activity was decreased only in striatum. However, it remains to be determined if mitochondrial dysfunction is rather a causal or a consequential event of abnormal signalling.

Cinara L. Gonçalves[1,2], Gislaine T. Rezin[1,2], Gabriela K. Ferreira[1,2], Isabela C. Jeremias[1,2], Mariane R. Cardoso[1,2], Milena Carvalho-Silva[1,2], Alexandra I. Zugno[2,3], João Quevedo[2,3], Emilio L. Streck[1,2]

[1]Laboratório de Bioenergética, Programa de Pós-graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil; [2]Instituto Nacional de Ciência e Tecnologia Translacional em Medicina, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil; and [3]Laboratório de Neurociências, Programa de Pós-graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil

Keywords: brain; creatine kinase; escitalopram; Krebs cycle; mitochondrial respiratory chain

Emilio L. Streck, Laboratório de Bioenergética, Universidade do Extremo Sul Catarinense, 88806-000 Criciúma, SC, Brazil.
Tel: +554834312539;
E-mail: emiliostreck@gmail.com

Accepted for publication June 25, 2011

Significant outcomes

- The inhibitory effect of escitalopram on respiratory chain complexes was different from other selective serotonin reuptake inhibitors (SSRIs), which usually increase the activity of these complexes.
- Escitalopram presents a specific effect on respiratory chain complexes, since the enzymes of Krebs cycle were not affected.

Limitations

- A complementary study to examine different doses in order to verify a possible dose-dependent effect of escitalopram.
- The effect of escitalopram in an animal model of depression should also be studied.

Introduction

Depressive disorders are serious and disabling, including major depression. This sort of depression is the most prevalent mental disorder which is estimated to be prevalent in 13.5–21.2% of the cases (1–3). The World Health Organisation estimates that major depression is the fourth most important cause of loss in disability-adjusted life years worldwide (4–6). Interpersonal relationships are affected by this disability to a larger extent; spouses and children are the ones impacted by this aspect of impaired social functioning. It also has high economic costs, both in terms of direct treatment costs and working days lost through illness (7). Along with schizophrenia, depression is responsible for 60% of suicides worldwide and it is predicted to be the second main cause of disability in 2020 in all patients regardless of their age and gender (8). Although it is believed that depression has genetic antecedents, which also suggests a biological contribution to its origin, the exact pathophysiology of depression is not clearly understood (9).

Current studies of pathophysiological mechanisms and the pharmacological treatment of major depressive disorder are emphasised mainly on the monoamine hypothesis (10). This theory suggests that major depression results from an unbalance of neurotransmission such as serotonin (5-HT), norepinephrine and dopamine, and the treatments are based on normalising the levels of these neurotransmitters (11).

The treatment of depression has focused on the neurotransmitter serotonin for the last decade. As tricyclic compounds have a less tolerable profile, SSRIs have been more widely prescribed for being safe and effective when compared with those older compounds (12). Escitalopram is functionally classified as an SSRI, which enhances serotonergic transmission by blocking the presynaptic active membrane transport mechanism for the reuptake of serotonin and consequently increases serotonergic activity at the postsynaptic receptor (13,14).

Microdialysis studies have shown that a single intraperitoneal (IP) injection of SSRIs increased the extracellular concentration of 5-HT in mice cortex (15). However, the data obtained from animal studies using antidepressants are contradictory and they still remain unclear whether these SSRIs show a true anxiogenic or anxiolytic effect or have no effect (16).

Studies have shown that mitochondrial dysfunction has been implicated in the pathogenesis of a number of diseases affecting the brain such as dementia, cerebral ischaemia, Alzheimer's disease and Parkinson's disease (17–23). Tissues with high-energy demands, such as the brain, contain a large number of mitochondria, which makes them more susceptible to reduction of the aerobic metabolism. Mitochondria are intracellular organelles which optimise adenosine triphosphate (ATP) production (24). Oxidative phosphorylation is the process in which most cell energy production is obtained. This process requires the action of various respiratory enzyme complexes located in a special structure of the inner mitochondrial membrane, the mitochondrial respiratory chain (25).

The Krebs cycle is the major, final, common pathway for oxidation of carbohydrates, lipids and some amino acids, which produces reducing equivalents in the form of nicotinamide adenine dinucleotide and flavin adenine dinucleotide that result in large production of ATP through oxidative phosphorylation (26). Some enzymes of Krebs cycle stand out with important roles. Citrate synthase (EC 4.1.3.7), an important regulatory step of Krebs cycle, is found within cells in the mitochondrial matrix and catalyses the condensation of oxaloacetate and the acetyl group of acetyl coenzyme-A (acetyl CoA) (27). Malate dehydrogenase (EC 1.1.1.37) is a key enzyme that plays an important metabolic function in aerobic energy-producing pathways and in the malate shuttle. It catalyses the dehydrogenation of L-malate to oxaloacetate at the end of Krebs cycle (26,28). Succinate dehydrogenase (SDH) (EC 1.3.99.1) is one of the most reliable indicator of the mitochondrial capability to supply an adequate amount of ATP, as it is part of both Krebs cycle and respiratory chain (complex II). SDH catalyses the oxidation of succinate to fumarate in the Krebs cycle and feed electrons to the respiratory chain ubiquinone (UQ) pool (29,30).

Creatine kinase (EC 2.7.3.2) takes part in metabolism of high-energy-consuming tissues such as brain, where it functions as an effective buffering system of cellular ATP levels. The enzyme catalyses the reversible transfer of the phosphoryl group from

https://doi.org/10.1111/j.1601-5215.2011.00592.x Published online by Cambridge University Press

phosphocreatine to adenosine diphosphate (ADP), regenerating ATP. It is also known that a reduction of creatine kinase activity might impair energy homeostasis, ending in cell death (31–35).

On the basis of the hypothesis that metabolism impairment might be involved in the pathophysiology of depression, in this study, we evaluated the activities of enzymes of Krebs cycle, mitochondrial respiratory chain complexes and creatine kinase in brain of rats subjected to acute administration of escitalopram.

Materials and methods

Animals

Adult and male Wistar rats (250–300 g) were obtained from Central Animal House of the Universidade do Extremo Sul Catarinense. They were caged in group of five with free access to food and water and were maintained on a 12-h light–dark cycle (lights on 7:00 h), at a temperature of 23 ± 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 recommendations for animal care, with the approval of the Ethics Committee from Universidade do Extremo Sul Catarinense.

Drugs

Animals received daily IP injections of escitalopram (10 mg/kg), in 1.0 ml/kg volume for 14 days ($n = 6$ animals per group). All drugs were dissolved in saline solution (vehicle). Control animals received only vehicle (1.0 ml/kg). The selection of this regimen was based on previous studies showing important neurochemical and antidepressant effects for this drug (36).

Tissue and homogenate preparation

Twelve hours after the last injection, the rats were killed by decapitation, the brain was removed and the prefrontal cortex, hippocampus, striatum, cerebellum and cerebral 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 longest 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 (37) using bovine serum albumin as standard. Biochemical preparation included mitochondria from different cells (neurons, astrocytes, microglia, etc.).

Activities of mitochondrial respiratory chain enzymes

NADH dehydrogenase (complex I) was evaluated by the method described by Cassina and Radi (38) 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 (complexes II–III) were determined by the method described by Fischer and colleagues (39). Complex II activity was measured by the monitoring of the decrease in absorbance due to the reduction of 2,6-DCIP at $\lambda = 600$ nm. The activity of complexes II–III 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 and colleagues(40), measured by the monitoring of the decrease in absorbance due to 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.

Activities of enzymes of Krebs cycle

Citrate synthase activity. Citrate synthase activity was assayed according to the method described by Shepherd and Garland (27). 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 (41). 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.

SDH activity. SDH activity was determined according to the method of Fischer and colleagues (39), measured by monitoring the decrease in absorbance due to the reduction of 2,6-di-chloro-indophenol (2,6-DCIP) at 600 nm with 700 nm as reference wavelength ($\varepsilon = 19.1\ mM^{-1}\ cm^{-1}$) 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

https://doi.org/10.1111/j.1601-5215.2011.00592.x Published online by Cambridge University Press

preincubated 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.

Activity of creatine kinase

Creatine kinase 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 (42). The colour 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 analysed by Student's t-test and were expressed as mean ± standard deviation. All analyses were performed using the Statistical Package for the Social Science (SPSS) software, IBM Corporation, New York, USA.

Results

This work investigated the activities of respiratory chain complex in the presence of escitalopram in homogenates from prefrontal cortex, hippocampus, striatum, cerebellum and posterior cortex from rat brain. Our results showed that rats treated with this antidepressant presented a significant decrease in complex I activity (Fig. 1a) in cerebellum, hippocampus and striatum, whereas prefrontal and

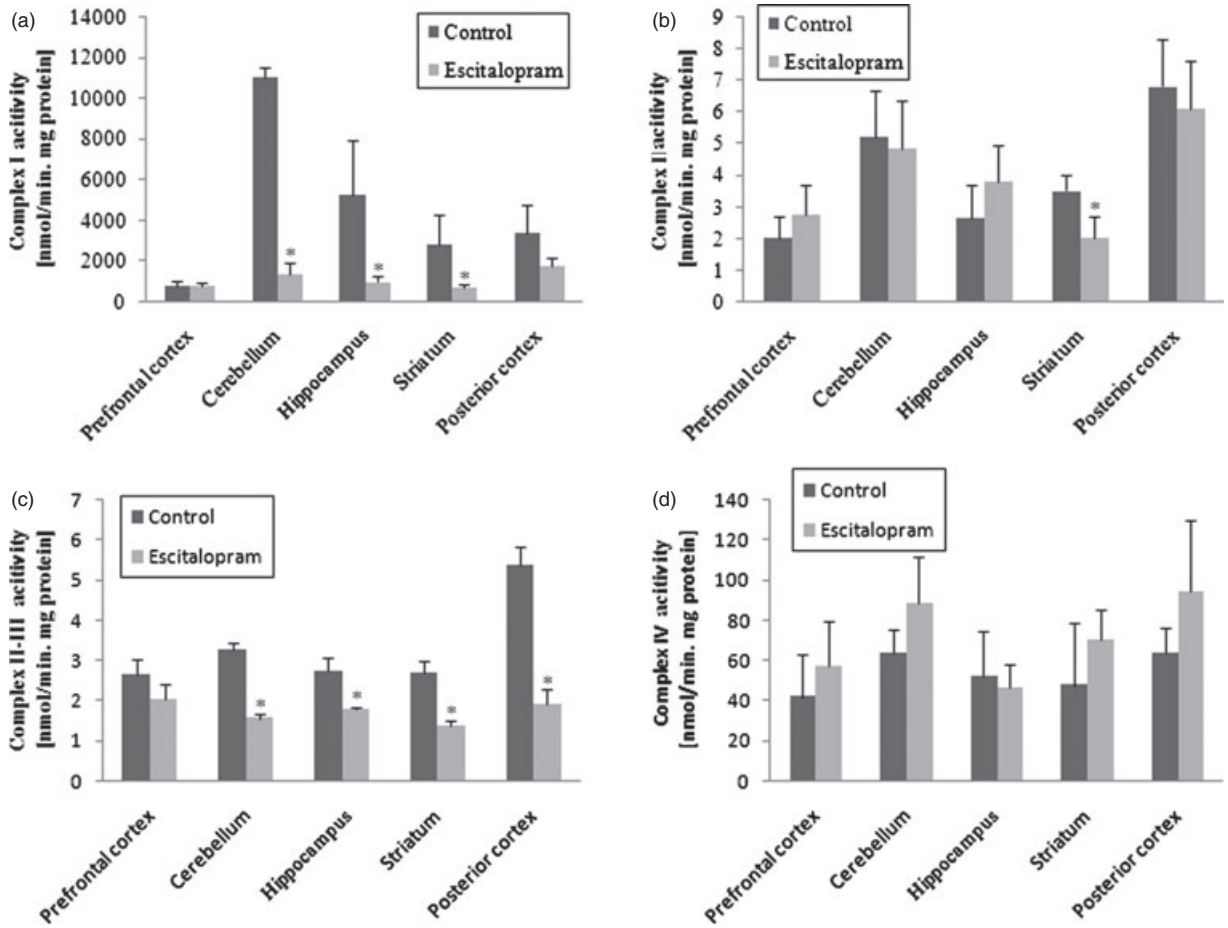

Fig. 1. Complex I activity (a), complex II activity (b), complex II–III activity (c) and complex IV activity (d) activity after chronic administration of escitalopram in the prefrontal cortex, cerebellum, hippocampus, striatum and cerebral cortex of rats. Data were analysed by the Student's t-test. Values are expressed as nmol/min × mg protein, mean ± SD ($n = 6$). *Different from control; $p < 0.05$.

https://doi.org/10.1111/j.1601-5215.2011.00592.x Published online by Cambridge University Press

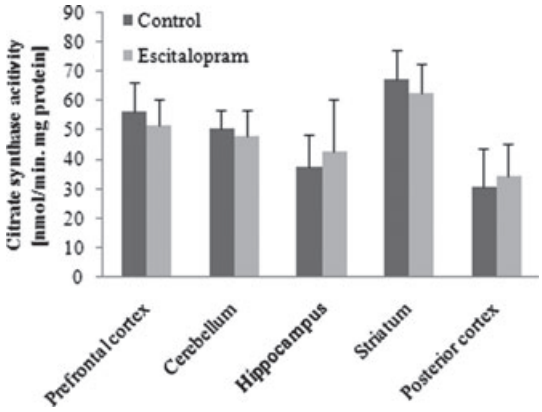

Fig. 2. Citrate synthase activity after chronic administration of escitalopram in the prefrontal cortex, cerebellum, hippocampus, striatum and posterior cortex of rats. Data were analysed by the Student's *t*-test. Values are expressed as nmol/min × mg protein, mean ± SD ($n = 6$). *Different from control; $p < 0.05$.

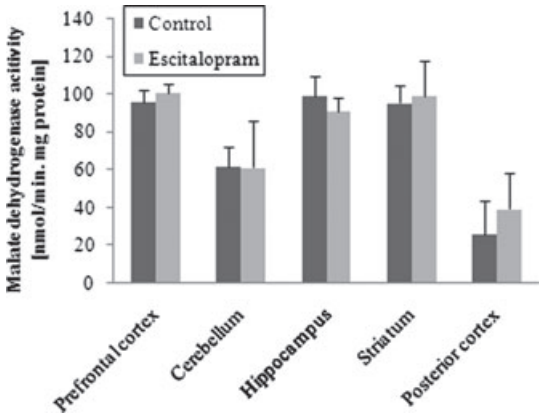

Fig. 3. Malate dehydrogenase activity after chronic administration of escitalopram in the prefrontal cortex, cerebellum, hippocampus, striatum and posterior cortex of rats. Data were analysed by the Student's *t*-test. Values are expressed as nmol/min × mg protein, mean ± SD ($n = 6$). *Different from control; $p < 0.05$.

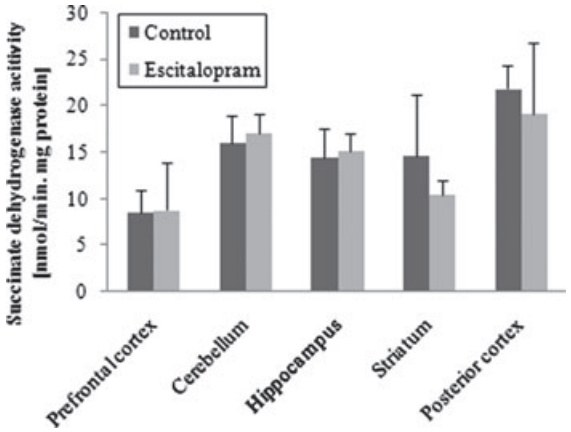

Fig. 4. SDH activity after chronic administration of escitalopram in the prefrontal cortex, cerebellum, hippocampus, striatum and posterior cortex of rats. Data were analysed by the Student's *t*-test. Values are expressed as nmol/min × mg protein, mean ± SD ($n = 6$). *Different from control; $p < 0.05$.

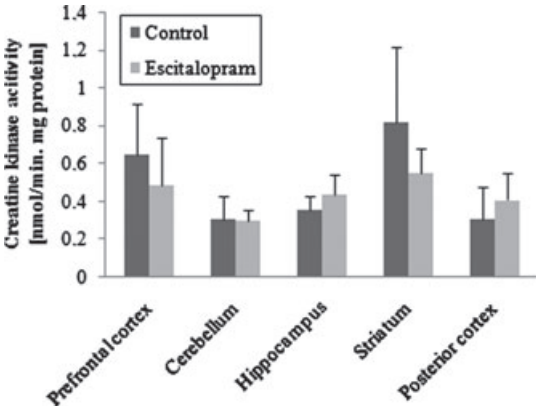

Fig. 5. Creatine kinase activity after chronic administration of escitalopram in the prefrontal cortex, cerebellum, hippocampus, striatum and cerebral cortex of rats. Data were analysed by the Student's *t*-test. Values are expressed as nmol/min × mg protein, mean ± SD ($n = 6$). *Different from control; $p < 0.05$.

posterior cortexes were not affected. Similar findings occurred with complex II–III activity (Figure 1c), where the same structures were affected plus posterior cortex. Furthermore, complex II activity (Fig. 1b) was decreased only in striatum without affecting prefrontal cortex, hippocampus, cerebellum and posterior cortex. On the other hand, chronic administration of escitalopram did not affect complex IV activity (Fig. 1d) as well as all other important analysed enzymes in the Krebs cycle (Figs 2–4) and creatine kinase (Fig. 5).

Discussion

Most recent findings suggest the possibility that a mitochondrial deficit is enough to trigger one or more psychiatric disorders (43). Moreover, several works also support the hypothesis that metabolism impairment is involved in the pathophysiology of depression (44–47). Madrigal and colleagues (48) showed that stress (immobilisation for 6 h during 21 days) inhibited the activities of complexes of the mitochondrial respiratory chain. An abnormal cellular energy state can lead to alteration in neuronal function, plasticity and brain circuit, and thereby to the cognition (49,50). We have recently showed that creatine kinase is inhibited in animal models of neuropsychiatry disorders such as bipolar disorder (51) and after electroconvulsive shock (52).

It has been also reported that brain Na^+, K^+-ATPase and activities of respiratory chain complexes I, III and IV are inhibited after chronic variate stress in rats (53,54) and that complexes I–III and II–III

https://doi.org/10.1111/j.1601-5215.2011.00592.x Published online by Cambridge University Press

of mitochondrial respiratory chain are inhibited in rat brain after chronic stress (50).

In brain structure samples analysed in this study, the most affected area was the striatum, with abnormal activity in complexes I, II, II–III. The striatum is a dopaminergic area involved in memory (55) and mood disorders (56). Additionally, striatum also has relation to the start, stop and direction of motor movement (57); in other areas investigated, hippocampus, cerebellum and posterior cortex, there was an alteration in the activity of complexes I and II–III. The hippocampus is one of several limbic structures that have been related to mood disorders. In addition, the hippocampus has connections with the prefrontal cortex, an area that is more directly involved in emotion and cognition and, thereby, contributes to other major symptoms of mood disorders (58–60).

In this study, we verified an unexpected decrease in some respiratory chain complexes from rat brain after chronic administration of escitalopram, especially in the activities of complexes I and II–III in most brain structures like cerebellum, hippocampus, striatum and posterior cortex. A recent study showed that citalopram, the isomer form of escitalopram, decreased the activity of respiratory chain complexes I and IV (61). This is a relevant concept for medications to be considered, although enantiomers differ only in how they rotate plane-polarised light, they may have very different biological properties (62). Various pre-clinical and clinical studies with escitalopram for the treatment of major depression have been conducted and supply further evidence of the potency and efficacy of escitalopram (63–65) between its peculiarities is more rapid onset of action when compared with citalopram. We verified that escitalopram, like citalopram, showed inhibitory effect on mitochondrial respiratory chain.

It is known that complex I is a key element in the control of oxidative phosphorylation and its abnormal activity can lead to defects in energy metabolism and changes in neuronal activity (66). In addition, complex I is the primary source of reactive oxygen species (ROS) in a variety of pathological processes (67,68). This tendency of xenobiotics to inhibit complex I (mitochondrial NADH:UQ oxidoreductase; EC 1.6.5.3) may depend on the structure of this enzymatic complex, which consists of at least 40 different polypeptides in the inner mitochondrial membrane. This unique feature explains the mitochondrion's great vulnerability to lipophilic molecules (69–71). Inhibition of complex III usually results in the generation of ROS as a consequence of the intrinsic characteristics of the electron-transfer process to this complex from reduced UQ (72).

Our study also showed that chronic treatment with escitalopram does not alter citrate synthase activity such as other enzymes of Krebs cycle, succinate dehidrogenase and malate dehydrogenase. Together, they play an important role in brain energy metabolism. It is important to consider that we have some limitations such as the time of administration of the drug in this study. Moreover, we observed only the effect of the drug *per se* and a different effect may be found if we test the drug in an animal model of variant chronic stress.

On the basis of the hypothesis that metabolism impairment might be involved in the pathophysiology of depression, our findings of mitochondrial changes induced by escitalopram support the idea that mitochondrial dysfunction could be a primary event. Escitalopram treatment affects the metabolic parameters of brain. Our findings suggest that therapeutic or side-effects of escitalopram may be due to its differential effects on the specific biochemical metabolic parameters of both cortical and subcortical brain regions.

Acknowledgements

This research was supported by grants from Conselho Nacional de Pesquisa e Desenvolvimento (CNPq), Fundação de Apoio à Pesquisa Científica e Tecnológica do Estado de Santa Catarina (FAPESC) and Universidade do Extremo Sul Catarinense (UNESC).

References

1. Kessler RC, Walters EE. Epidemiology of DSM-III-R major depression and minor depression among adolescents and young adults in the National Comorbidity Survey. Depress Anxiety 1998;**7**:3–14.
2. Turner JR, Gil AG. Psychiatric and substance use disorders in South Florida racial/ethnic and gender contrasts in a young adult cohort. Arch Gen Psychiatry 2002;**59**:43–50.
3. Kessler RC, Berglund P, Demler O, Jin R, Merikans KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorder in the national comorbidity survey replication. Arch Gen Psychiatry 2005;**62**:593–768.
4. Rex A, Schickert R, Fink H. Antidepressant-like effect of nicotinamide adenine dinucleotide in the forced swim test in rats. Pharmacol Biochem Behav 2004;**77**:303–307.
5. Kiss JP. Theory of active antidepressants: a nonsynaptic approach to the treatment of depression, Neurochem Int 2008;**52**:34–39.
6. Longone P, Rupprecht R, Manieri GA, Bernardi G, Romeo E, Pasini A. The complex roles of neurosteroids in depression and anxiety disorders. Neurochem Int 2008;**52**:596–601.
7. Greenberg PE, Stiglin LE, Finkelstein SN, Berndt ER, 1993. The economic burden of depression in 1990. J Clin Psychiatry 1990;**54**:405–418.
8. World Health Organization: Programmes and projects/mental health/disorders management/depression. Retrieved March 1, 2010, from URL http:// www.who.int/mental_health/management/depression/definition/en/

https://doi.org/10.1111/j.1601-5215.2011.00592.x Published online by Cambridge University Press

9. Cannon TD, Keller MC. Endophenotypes in the genetic analyses of mental disorders. Annu Rev Clin Psychol 2006;**2**:267–290.
10. Skolnick P. Beyond monoamine-based therapies: clues to new approaches. J Clin Psychiatry 2002;**63**:19–23.
11. Charney DS. Monoamine dysfunction and the pathophysiology and treatment of depression. J Clin Psychiatry 1998;**59**:11–14.
12. Brunello N, Mendlewicz J, Kasper S et al. The role of noradrenaline and selective noradrenaline reuptake inhibition in depression. Eur Neuropsychopharmacology 2002;**12**:461–475.
13. Johnson AM. Paroxetine: a pharmacological review. Int Clin Psychopharmacol 1992;**6**:15–24.
14. Richelson E. Pharmacology of anti-depressants – characteristics of the ideal drug. Mayo Clin Proc 1999;**69**: 1069–1081.
15. David DJ, Bourin M, Jego G, Przybylski C, Jolliet P, Gardier AM. Effects of acute treatment with paroxetine, citalopram and venlafaxine in vivo on noradrenaline and serotonin outflow: a microdialysis study in Swiss mice. Br J Pharmacol 2003;**140**:1128–1136.
16. Borsini F, Podhorna J, Marazziti D. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? Psychopharmacology 2002;**163**:121–141.
17. Brennan WA, Bird ED, Aprille JR. Regional mitochondrial respiratory activity in Huntington's disease brain. J Neurochem 1985;**44**:1948.
18. Heales SJ, Bolanõs JP, Stewart VC, Brookes PS, Land JM, Clark JB. Nitric oxide, mitochondria and neurological disease. Biochim Biophys Acta 1999;**1410**: 215–228.
19. Blass JP. Brain metabolism and brain disease: is metabolic deficiency the proximate cause of Alzheimer dementia? J Neurosci Res 2001;**66**:851–856.
20. Schurr A. Energy metabolism, stress hormones and neural recovery from cerebral ischemia/hypoxia. Neurochem Int 2002;**41**:1–8.
21. Monsalve M, Borniquel S, Valle I, Lamas S. Mitochondrial dysfunction in human pathologies. Front Biosci 2007;**12**:1131–1153.
22. Moreira PI, Santos MS, Oliveira CR. Alzheimer's disease: a lesson from mitochondrial dysfunction. Antioxid Redox Signal 2007;**9**:1621–1630.
23. Moreira PI, Santos MS, Seiça R, Oliveira CR. Brain mitochondrial dysfunction as a link between Alzheimer's disease and diabetes. J Neurol Sci 2007;**257**:206–214.
24. 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.
25. Boekema EJ, Braun HP. Supramolecular structure of the mitochondrial oxidative phosphorylation system. J Biol Chem 2007;**282**:1–4.
26. Kelly D, Gordon J, Alpers R, Strauss AW. The tissue-specific expression and developmental regulation of two nuclear genes encoding rat mitochondrial proteins. Medium chain acyl-CoA dehydrogenase and mitochondrial malate dehydrogenase. J Biol Chem 1989;**264**:18921–18925.
27. Shepherd D, Garland PB. The kinetic properties of citrate synthase from rat liver mitochondria. Biochem J 1969;**114**:597–610.
28. Labrou NE, Clonis YD. L-Malate dehydrogenase from *Pseudomonas stutzeri*: purification and characterization. Arch Biochem Biophys 1997;**337**:103–114.
29. Tyler D. The mitochondrion in health and diseases. New York: VCH Publishers, 1992.
30. Rustin P, Munnich A, Rötig A. Succinate dehydrogenase and human diseases: newinsights into a well-known enzyme. Eur J Hum Genet 2002;**10**:289–291.
31. 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.
32. Hamman BL, Bittl JA, Jacobus WE et al. Inhibition of the creatine kinase reaction decreases the contractile reserve of isolated rat hearts. Am J Physiol 1995;**269**:1030–1036.
33. Gross WL, Bak MI, Ingwall JS et al. Nitric oxide inhibits creatine kinase and regulates heart contractile reserve. Proc Natl Acad Sci U S A 1996;**93**:5604–5609.
34. 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.Brain Res Mol Brain Res 1998;**54**:276–287.
35. 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.
36. Jayatissa MN, Bisgaard C, Tingström A, Papp M, Wiborg O. Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. Neuropsychopharmacology 2006;**31**: 2395–2404.
37. Lowry OH, Rosebough NG, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;**193**:265–275.
38. Cassina A, Radi R. Differential inhibitory Aation of nitric oxide and peroxynitrite on mitochondrial electron transport. Arch Biochem Biophys 1996;**328**:309–316.
39. Fischer JC, Ruitenbeek W, Berden JA, Trijbels JM. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. Clin Chim Acta 1985;**153**:23–26.
40. Rustin P, Chretien D, Bourgeron T et al. Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta 1994;**228**:35–51.
41. Kitto GB. Intra- and extramitochondrial malate dehydrogenases from chicken and tuna heart. Methods Enzymol 1969;**8**:106–116.
42. 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.
43. Mattson MP, Gleichmann M, Cheng A. Mitochondria in neuroplasticity and neurological disorders. Neuron 2008;**60**:748–766.
44. Retter L, Mayer-Takacs D, Adam-Vizi V. The effect of bovine serum albumin on the membrane potential and reactive oxygen species generation in succinate-supported isolated brain mitochondria. Neurochem Int 2007;**50**:139–147.
45. Petrosillo G, Matera M, Casanova G, Ruggiero FM, Paradies G. Mitochondrial dysfunction in rat brain with aging: involvement of complex I, reactive oxygen species and cardiolipin. Neurochem Int 2008;**53**:126–131.

https://doi.org/10.1111/j.1601-5215.2011.00592.x Published online by Cambridge University Press

46. Kanarik M, Matrov D, Kõiv K, Eller M, Tõnissaar M, Harro J. Changes in regional long-term oxidative metabolism induced by partial serotonergic denervation and chronic variable stress in rat brain. Neurochem Int 2008;**52**:432–437.
47. Stanyer L, Jorgensen W, Hori O, Clark JB, Heales SJ. Inactivation of brain mitochondrial Lon protease by peroxynitrite precedes electron transport chain dysfunction. Neurochem Int 2008;**53**:95–101.
48. Madrigal JL, Olivenza R, Moro M et al. Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain. Neuropsychopharmacology 2001;**24**:420–429.
49. Barrett SL, Kelly C, Bell R, King DJ. Gender influences the detection of spatial working memory deficits in bipolar disorder. Bipolar Disord 2008;**10**:647–654.
50. Quraishi S, Walshe M, Mcdonald C et al. Memory functioning in familial bipolar I disorder patients and their relatives. Bipolar Disord 2009;**11**:209–214.
51. Streck EL, Amboni G, Scaini G et al. Brain Creatine kinase activity in an animal model of mania. Life Sci 2008;**82**:424–429.
52. Burigo M, Roza CA, Bassani C et al. Decreased Creatine kinase activity caused by electroconvulsive shock. Neurochem Res 2006;**31**:877–881.
53. Gamaro GD, Streck EL, Matté C, Prediger ME, Wyse AT, Dalmaz C. Reduction of hippocampal Na^+, K^+-ATPase activity in rats subjected to an experimental model of depression. Neurochem Res 2003;**28**:1339–1344.
54. Rezin GT, Cardoso MR, Gonçalves CL et al. Inhibition of mitochondrial respiratory chain in brain of rats subjected to an experimental model of depression. Neurochem Int 2008;**53**:395–400.
55. Ferretti V, Roullet P, Sargolini F et al. Ventral striatal plasticity and spatial memory. Proc Natl Acad Sci U S A 2010;**107**:7945–7950.
56. Marais L, Stein DJ, Daniels WM. Exercise increases BDNF levels in the striatum and decreases depressive-like behavior in chronically stressed rats. Metab Brain Dis 2009;**24**:587–597.
57. Gokce O, Runne H, Kuhn A, Luthi-Carter R. Short-term striatal gene expression responses to brain-derived neurotrophic factor are dependent on MEK and ERK activation. PLoS One 2009;**4**:5292.
58. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biol Psychiatry 2006;**59**: 1116–1127.
59. Hall J, Whalley HC, Marwick K et al. Hippocampal function in schizophrenia and bipolar disorder. Psychol Med 2009;**7**:1–10.
60. Hassel S, Almeida JR, Kerr N et al. Elevated striatal and decreased dorsolateral prefrontal cortical activity in response to emotional stimuli in euthymic bipolar disorder: no associations with psychotropic medication load. Bipolar Disord 2008;**10**:916–927.
61. Hroudova J, Fisar Z. Activities of respiratory chain complexes and citrate synthase influenced by pharmacologically different antidepressants and mood stabilizers. Neuroendocrinology Lett 2010;**31**:336–342.
62. Burke WJ, Kratochvil CJ. Stereoisomers in psychiatry: the case of escitalopra. Prim Care Companion J Clin Psychiatry 2002;**4**:20–24.
63. Mitchell PJ, Hogg S. Beavioural effects of escitalopram predict potent antidepressant activity. Presented at the 7th World Congress of Biological Psychiatry, July 1–6. Berlin, Germany, 2001.
64. Sanchez C, Hogg S. The antidepressant effect of citalopram resides in the S-enantiomer (Lu 26-054). Presented at the 55th annual meeting of the Society of Biological Psychiatry, May 11–13. Chicago, IL, 2000.
65. Mitchell P, Hogg S. Escitalopram: rapid antidepressant activity in rats. Presented at the 7th World Congress of Biological Psychiatry, July 1–6. Berlin, Germany, 2001.
66. Pathak RU, Davey GP. Complex I and energy theresholds in the brain. Biochim Biophys Acta 2008;**1777**: 777–782.
67. Barja G, Herrero AJ. Localization at complex I and mechanism of the higher free radical production of brain nonsynaptic mitochondria in the short-lived rat than in the longevous pigeon. J Bioenerg Biomembr 1998;**30**:235–224.
68. Sherer TB, Betarbet R, Kim JH, Greenamyre JT. Selective microglial activation in the rat rotenone model of Parkinson's disease. Neurosci Lett 2003;**341**:87–90.
69. Degli EM. Inhibitors of NADH-ubiquinone reductase: an overview. Biochim Biophys Acta 1998;**1364**:222–235.
70. Miyoshi H. Structure-activity relationships of some complex I inhibitors. Biochim Biophys Acta 1998;**1364**: 236–244.
71. Don AS, Hogg PJ. Mitochondria as cancer drug targets. Trends Mol Med 2004;**10**:372–378.
72. Scatena R, Bottoni P, Botta G, Martorana GE, Giardina B. The role of mitochondria in pharmacotoxicology: a reevaluation of an old, newly emerging topic. Am J Physiol Cell Physiol 2007;**293**:C12–C21.

https://doi.org/10.1111/j.1601-5215.2011.00592.x Published online by Cambridge University Press