

Rivastigmine reverses cognitive deficit and acetylcholinesterase activity induced by ketamine in an animal model of schizophrenia

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Received: 22 January 2013 / Accepted: 28 May 2013 / Published online: 19 June 2013
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Abstract Schizophrenia is one of the most disabling mental disorders that affects up to 1 % of the population worldwide. Although the causes of this disorder remain unknown, it has been extensively characterized by a broad range of emotional, ideational and cognitive impairments. Studies indicate that schizophrenia affects neurotransmitters such as dopamine, glutamate and acetylcholine. Recent studies suggest that rivastigmine (an acetylcholinesterase inhibitor) is important to improve the cognitive symptoms of schizophrenia. Therefore, the present study evaluated the protective effect of rivastigmine against the ketamine-induced behavioral (hyperlocomotion and cognitive deficit) and biochemical (increase of acetylcholinesterase activity) changes which characterize an animal model of schizophrenia in rats. Our results indicated that rivastigmine was effective to improve the cognitive deficit in different task (immediate memory, long term memory and short term memory) induced by ketamine in rats. Moreover, we observed that rivastigmine reversed the increase of acetylcholinesterase activity induced by ketamine in the cerebral cortex, hippocampus and striatum. However, rivastigmine was not able to prevent the ketamine-induced hyperlocomotion. In conclusion, ours

results indicate that cholinergic system might be an important therapeutic target in the physiopathology of schizophrenia, mainly in the cognition, but additional studies should be carried.

Keywords Schizophrenia · Ketamine · Memory · Acetylcholinesterase · Locomotor activity · Rivastigmine

Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
ChAT	Choline acetyltransferase
ChEI	Acetylcholinesterase inhibitors
CNS	Central nervous system
NMDA	N-Methyl-D-aspartate

Introduction

Schizophrenia is one of the most severe and disabling psychiatric disorders that affects up to 1 % of the population worldwide and it causes an enormous social and economic impact (Mueser and Penn 2004). The physiopathology of the disease is poorly understood and is usually associated with the incidence of comorbidities. The affected individuals have a profound disturbance of mental functions, emotions and behavior, which disrupts many of the most basic human processes of perception and judgment. The clinical symptoms include positive symptoms such as hallucinations and delusions, negative symptoms as anhedonia, avolition and social withdrawal and cognitive symptoms like memory and attention impairment (Meyer and Feldon 2010). Currently, pharmacological therapy with antipsychotics is able to control the positive and negative symptoms, however the effects are still limited regarding cognitive function. Furthermore, most drugs generates neurological and physical side effects

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which hinders the adherence to the treatment and consequently induces relapses of symptoms (Schultz et al. 2007).

Dopamine plays an important role in the schizophrenia and almost all antipsychotics alter dopaminergic neurotransmission (Fredman, 20003). However, dopaminergic system cannot entirely explain the pathophysiology of schizophrenia, and the role of other neurotransmitters, such as acetylcholine (ACh), has been investigating (Schultz et al. 2007).

Acetylcholine (ACh) is a neurotransmitter that acts at both peripheral and central nervous system (CNS) of many organisms. The signaling of ACh modulates synaptic plasticity, particularly when related to learning and short-term memory (Woolf and Butcher 1986; Perry et al. 1999). This neurotransmitter is hydrolysed into synaptic cleft by acetylcholinesterase (AChE), an enzyme which is a key regulator of cholinergic transmission (Azevedo et al. 2011). Also, alterations in ACh levels are related with cognitive dysfunction in schizophrenia (Meltzer and McGurk 1999). In this context, some studies indicate that nicotine (agonist of nicotinic ACh receptor) improved the negative symptoms and cognitive performance in patients with schizophrenia (Barr et al. 2008; Jubelt et al. 2008). This fact possibly justifies the higher rates of smoking in this population comparing to general subjects (Hughes et al. 1986). Therefore, one of the challenges of the studies related to schizophrenia is to perform clinical research in order to improve the characteristics of the crippling disease, as well as the development of experimental research with the objective to find the best therapeutic targets.

Acetylcholinesterase inhibitors (ChEI) are useful for improving cognitive function in Alzheimer disease by increasing ACh levels into synaptic cleft. These drugs are also being tested in schizophrenic patients for cognitive improvement in schizophrenia (Ribeiz et al. 2010). Furthermore, several ChEI are available for clinical trials as tacrine, donepezil, rivastigmine and galantamine and they are also effective in Alzheimer's disease (Rogers et al. 1998; Rogers and Kashima 1998; Rösler et al. 1999; Tariot et al. 2000). In particular, some studies indicate that rivastigmine may be an important therapeutic strategy to improve the cognitive deficits in schizophrenic patients, but confirmatory studies are needed to determine the clinical utility of this drug (Ribeiz et al. 2010).

Animal models provide an opportunity to discover new targets for the treatment of schizophrenia. Accordingly, the animal model of ketamine is one of the most widely accepted since its generates behavioral (schizophrenia-like positive, negative and cognitive symptoms) and biochemical alterations in animals, similar to human schizophrenia (Becker et al. 2003; Chatterjee and Khanna 2011; Adell et al. 2012; Gilmour et al. 2012). Take into account that N-Methyl-D-aspartate (NMDA) receptor hypofunction is observed in the schizophrenia (Olney et al. 1999), this animal model induces similar alteration, since ketamine is a non-competitive antagonist of NMDA receptor,

an ionotropic glutamate receptor (Bressan and Pilowsky 2003; Adell et al. 2012; Gilmour et al. 2012). Many studies showed that ketamine administration induces schizophrenia-like behavioral changes (hyperlocomotion, deficit cognitive, stereotype) in rodents, indicating a useful animal model of schizophrenia (Becker et al. 2003; Becker and Grecksch 2004; de Oliveira et al. 2009; de Oliveira et al. 2011; Canever et al. 2010; Fraga et al. 2011; Chatterjee and Khanna 2011; Adell et al. 2012; Gilmour et al. 2012).

Considering that: (i) Schizophrenia is a psychiatric disorder complex, (Meyer and Feldon 2010), (ii) The discovery of new non-dopaminergic targets effective in reducing the symptoms is needed (Kane and Correll 2010), (iii) Ketamine administration induces schizophrenia-like behavioral Alterations in rats (Becker et al. 2003; Becker and Grecksch 2004; Oliveira et al. 2009; Canever et al. 2010; Chatterjee et al. 2,011), (iv) There is evidence that rivastigmine may be an important therapeutic target for improvement of cognitive function in schizophrenic patients (Ribeiz et al. 2010), we induced an animal model of schizophrenia through chronic administration of ketamine in sub-anesthetic doses and test different behavioral parameters in rats treated with rivastigmine.

Materials and methods

Animals

Adult male Wistar rats (250–300 g) were obtained from the Central Animal House of Universidade do Extremo Sul Catarinense (UNESC). 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 at 7:00 am), 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 (SBNeC) recommendations for animal care, with the approval of UNESC Ethics Committee.

Drugs

Ketamine (CU Chemie Uetikon, Germany), Rivastigmine (Novartis Pharma, Brazil) were directly dissolved in saline (Sal) solution (NaCl 0.9 %, w/v). The doses of ketamine (25 mg/kg, i.p., once a day for 14 days) and rivastigmine (0.5 mg/kg, i.p., once a day for 7 days) were based on previous studies performed by de Oliveira et al. (2011) and Comim et al. (2009), respectively.

Animal model of schizophrenia

The animals received one injection of ketamine (CU Chemie Uetikon, Germany) 25 mg/kg (i.p., once a day) or saline

(vehicle) for 14 days. Between the 8th and the 14th day, the animals received rivastigmine (0.5 mg/kg, i.p.) or saline (vehicle), that is, during this period two types of treatment were performed in animals. This experimental design led four experimental groups, as detailed below: saline/saline; saline/rivastigmine; ketamine/saline; ketamine/rivastigmine. Rivastigmine was administered 2 h after ketamine to prevent interaction between the drugs.

Behavioral tests were done on the 15th, 16th and 17th days. The animals received a single injection of ketamine or saline and locomotor activity (after 30 min from the injection) was assessed during 15 min using a proper apparatus. These rats were killed by decapitation immediately after the locomotor activity and prefrontal cortex, hippocampus and striatum were manually dissected on ice, rapidly frozen on dry ice and stored at -70°C for posterior analysis of acetylcholinesterase activity. On the 16th day (24 h after the last injection of ketamine or saline), an independent group of animals were used for evaluate the behavioral in the inhibitory avoidance task (16th and 17th). For a better understanding of the experimental design see Fig. 1.

Open-field test

We assessed locomotor activity using the open-field test. The test was performed in a $50 \times 25 \times 50$ cm arena and locomotor activity was measured for 15 min using a computerized system (Activity Monitor—Insight laboratory equipment, Ribeirão Preto, SP). This equipment monitored locomotor activity via the covered distance (cm) by the animal, dividing the evaluation time into blocks of 5 min. Each animal was constantly monitored by a system installed in the arena containing six parallel bars, each of which contained 16

infrared sensors that detect the rat's exact position and movement. This system allows a detailed analysis of each animal's behavior. Information detected by the sensors is transmitted to a computer in which the animal's activity is recorded each 5 min by a dedicated program (database: Open Source version Interbase 6.01). The covered distance by the animal is considered to be the sum of the changes in position monitored by the activity arena; the software calculates the distance between two locations, plus the previously traveled distances.

Inhibitory avoidance

The inhibitory avoidance evaluation was initiated 24 h after the last injection, with 60 rats, divided into 4 groups (saline/saline; saline/rivastigmine; ketamine/saline; ketamine/rivastigmine). The apparatus consisted of an acrylic box with parallel stainless steel bars and a platform placed on the floor (Quevedo et al. 1997; Roesler et al. 2003). In the training session rats were placed on the platform and we measured their latency to step down with all four paws. Immediately after step down from the platform, the animals received a 0.4 mA footshock (electrical shock induced through the feet) for 2 s.

In the test session, animals were again placed on the platform and had their latency to step down on the grid measured, except that no footshock was given. Latency is a classic parameter for memory retention tasks. Test sessions for inhibitory avoidance were conducted immediately after training (5 s) to evaluate immediate memory (Barros et al. 2005). The given interval between training and test sessions was 1.5 h to evaluate short-term memory (Izquierdo and Medina 1998; Bevilaqua et al. 2003) and 24 h to assessed long-term memory (Bevilaqua et al. 2003; Lima et al. 2005).

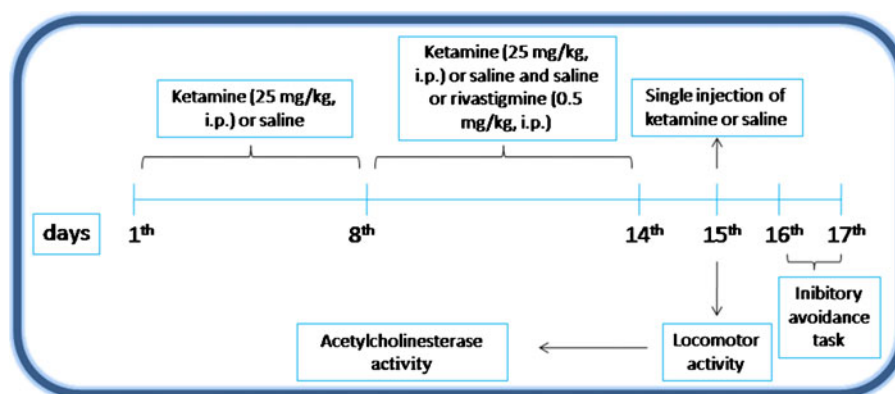


Fig. 1 Diagram of the complete experimental schedule. The animals received one injection of ketamine 25 mg/kg (i.p., once a day) or saline (vehicle) for 14 days. Between the 8th and the 14th day, the animals received rivastigmine (0.5 mg/kg, i.p., once a day for 7 days) or saline (vehicle). On the 15th day, the animals received a single injection of ketamine or saline and 30 min after this treatment, the locomotor activity

was assessed using the locomotor activity chamber for 15 min. Immediately after locomotor activity, these rats were killed by decapitation and prefrontal cortex, hippocampus and striatum were removed for analyses of acetylcholinesterase activity. An independent group of animals (24 h of last injection of ketamine or saline) was used for evaluate the behavioral in the inhibitory avoidance task (16th and 17th)

Acetylcholinesterase activity

After the evaluation of locomotor activity, animals were killed by decapitation and brain structures were rapidly dissected. Prefrontal cortex, striatum and hippocampus were homogenate and prepared to the assay.

AChE activity was assayed according to the method of Ellman et al. (1961). The reaction mixture (2 ml final volume) contained 100 mM K⁺-phosphate buffer (pH 7.5) and 1 mM 5,5'-dithiobisnitrobenzoic acid. The method is based on the formation of the yellow anion, 5,5'-dithio-bis-acid-nitrobenzoic, which is measured by absorbance at 412 nm during a 2-min incubation at 25 °C. The enzyme (40–50 µg of protein) was preincubated for 2 min. The reaction was initiated by adding 0.8 mM acetylthiocholine iodide (AcSCh). All samples were run in duplicate or triplicate, and the enzyme activity is expressed in micromoles AcSCh per hour per milligram of protein.

Protein determination

Protein was measured using the method described by Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

All experimental results are given as the mean±SEM. Comparisons between experimental and control groups were performed by two-way ANOVA followed by Newman-Keuls test when appropriate. A value of $p < 0.05$ was considered to be significant. Training-test session latency differences were assessed by the Wilcoxon test followed by individual Mann–Whitney *U*-test. A value of $p < 0.05$ was considered to be significant.

Results

Figure 2 shows the results related the memory of animals. We tested immediate memory, short and long-term memory consolidation. Statistical analysis indicates that no differences among groups were observed in training session. Differently, saline (control) or rivastigmine treated rats showed improvement of test session latency, when compared to training session, indicating that animals adequately learned the task (Wilcoxon test, $p < 0.05$). Ketamine impaired memory in all test session performance, as showed by Mann–Whitney *U*-tests, $p < 0.05$, (Fig. 2 [immediate memory, Mann–Whitney $U = 0.5$, $p < 0.001$; short term memory, Mann–Whitney $U = 12$, $p < 0.001$; long term memory, Mann–Whitney $U = 37.5$, $p < 0.001$]) when compared with control group.

In respect to locomotor activity, the animals treated with rivastigmine alone, for 7 days, did not show alteration of

covered distance. Animals that received ketamine injections for fourteen consecutive days presented a significant increase in locomotor activity when compared to animals that received saline. However, animals treated with ketamine+rivastigmine presented an increase in locomotor activity showing that rivastigmine has no effect on this parameter (Fig. 3). The two-way ANOVA revealed a significant effect of ketamine pretreatment [$F_{(1,56)} = 38.31$, $p < 0.01$], but not of rivastigmine treatment [$F_{(1,56)} = 0.27$, $p = 0.60$] and interaction between rivastigmine vs. ketamine [$F_{(1,56)} = 0.31$, $p = 0.58$].

We also studied the effect of ketamine and rivastigmine on AChE activity in the prefrontal cortex, hippocampus and striatum. As we already expected, rivastigmine alone inhibited AChE activity and ketamine significantly increased the enzyme activity. It was also observed that rivastigmine could reverse the effect caused by ketamine, as compared to control group (Fig. 4). The two-way ANOVA revealed a significant effect in the prefrontal cortex (ketamine pretreatment [$F_{(1,12)} = 68.19$, $p < 0.01$], rivastigmine treatment [$F_{(1,12)} = 61.86$, $p < 0.01$] and interaction between pretreatment vs. treatment [$F_{(1,12)} = 8.35$, $p < 0.05$]), hippocampus (ketamine pretreatment [$F_{(1,16)} = 109.15$, $p < 0.01$], rivastigmine treatment [$F_{(1,16)} = 82.64$, $p < 0.01$] and interaction between pretreatment vs. treatment [$F_{(1,16)} = 11.60$, $p < 0.01$]) and striatum (ketamine pretreatment [$F_{(1,15)} = 49.50$, $p < 0.01$], rivastigmine treatment [$F_{(1,15)} = 55.21$, $p < 0.01$] and interaction between pretreatment vs. treatment [$F_{(1,15)} = 6.13$, $p < 0.05$]).

Discussion

The results of present study showed that the rivastigmine was effective to improve the cognitive deficit in different task (immediate memory, long term memory and short term memory) induced by ketamine in rats. Moreover, we observed that the increase of acetylcholinesterase (AChE) activity induced by ketamine in the prefrontal cortex, hippocampus and striatum, was reversed by rivastigmine. However, rivastigmine was not able to prevent the ketamine-induced hyperlocomotion.

Glutamatergic (NMDA receptors) hypofunction induced by ketamine (non-competitive NMDA receptor antagonist) is an important animal model of schizophrenia, which have been utilized to try to understand this complex disease and to evaluate the therapeutic potential of candidate target for antipsychotics. This animal model induces positive (hyperlocomotion and stereotype), negative (social deficits) and cognitive (cognitive deficit) symptoms (Neill et al. 2010; Adell et al. 2012; Gilmour et al. 2012). Those results were also observed by our group and other researches group (Becker et al. 2003; Becker and Grecksch 2004; Chatterjee and Khanna 2011). In agreement, in this work, ketamine induced hyperlocomotion, cognitive deficit and biochemical alterations (de Oliveira et al.

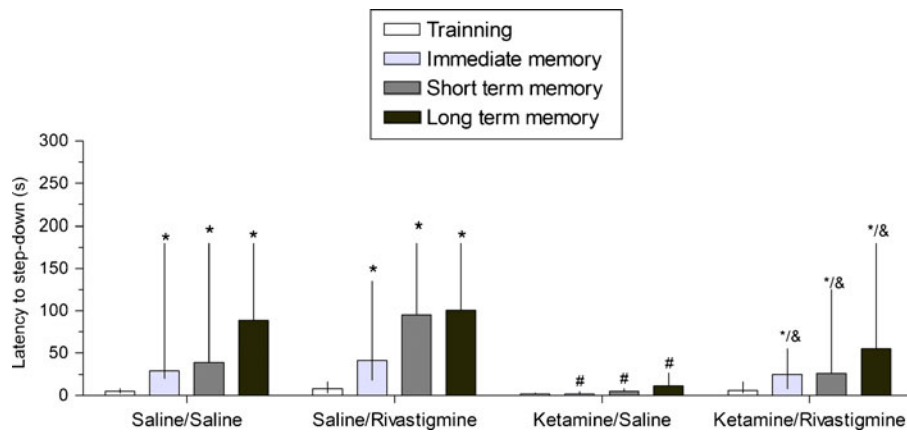


Fig. 2 Effect of the rivastigmine (0.5 mg/kg, i.p./day for 7 days) on different task of memory (immediate memory, short and long term memory) in a ketamine-induced animal model of schizophrenia (25 mg/kg, i.p./day for 7 days) in rats. Data are expressed as median±interquartile

range, $n=15$. * $P<0.05$ when compared to training of all experimental groups. # $P<0.05$ when compared to control group (Saline/Saline) of each type of memory, respectively. & $P<0.05$ when compared to ketamine/saline group of each type of memory, respectively

2009; de Oliveira et al. 2011; Canever et al. 2010; Fraga et al. 2011).

Although dopamine has been considered the key neurotransmitter involved in the pathophysiology of schizophrenia (Barch and Ceaser 2012; Howes et al. 2012), many studies have established that the cholinergic system (muscarinic and nicotinic receptors) is important for neuromodulation of cognitive processes in schizophrenia, since this disorder is associated with a range of cognitive deficits (Davis et al. 1975; Powchik et al. 1998; Sarter et al. 2012). Furthermore, it is well known that classical antipsychotics do not improve the cognitive deficits in both schizophrenic patients and pre-clinical models of schizophrenia (Neill et al. 2010). Therefore, in the present study we used the rivastigmine, an AChE inhibitor widely used in the treatment of Alzheimer disease, for evaluating the possible beneficial effect in enhancing cognitive functions in the animal model of schizophrenia induced by ketamine in rats.

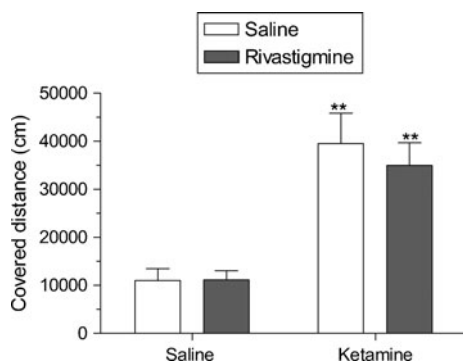


Fig. 3 Effect of the rivastigmine (0.5 mg/kg, i.p./day for 7 days) on locomotor activity in a ketamine-induced animal model of schizophrenia (25 mg/kg, i.p./day for 7 days) in rats. Data were analyzed by two-way ANOVA followed by Newman-Keuls test when appropriated. Values are expressed as mean±S.E.M. ($n=15$). ** $P<0.01$ when compared to saline/saline group

The results from behavior analysis indicate that rivastigmine did not reverse the ketamine-induced hyperlocomotion. These results indicated that the behavioral effect of rivastigmine is specific to cognition (cognitive symptoms of schizophrenia), but not to locomotion (positive symptoms of schizophrenia). It is important emphasize that the cholinergic system is closely involved with the cognition, since that drugs

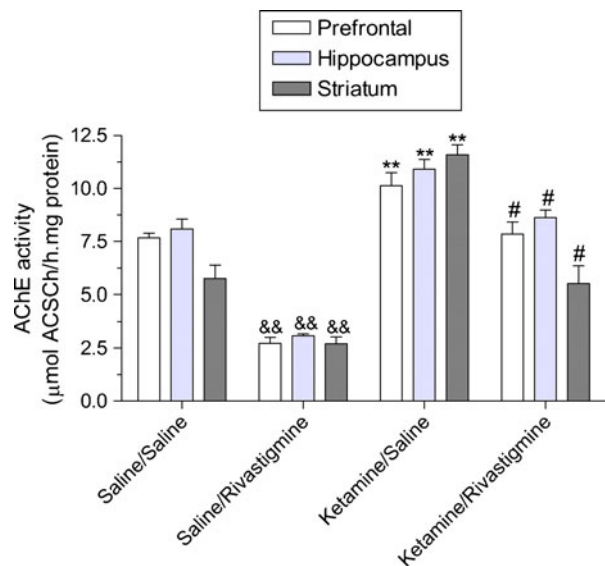


Fig. 4 Effect of the rivastigmine (0.5 mg/kg, i.p./day for 7 days) on acetylcholinesterase (AChE) activity in a ketamine-induced animal model of schizophrenia (25 mg/kg, i.p./day for 7 days) in prefrontal cortex, hippocampus and striatum of rats. Data were analyzed by two-way ANOVA followed by Newman-Keuls test when appropriated. Values are expressed as mean±S.E.M. ($n=4-6$) && $P<0.01$ when compared to saline/saline group, it is indicating decrease when compared to saline/saline group. ** $P<0.01$ when compared to saline/saline group, it is indicating increase when compared to saline/saline group. # $P<0.01$ compared to ketamine/saline group, it is indicating reversion when compared to ketamine/saline group

that enhance cholinergic neural transmission are well known for pro-cognitive properties (Singh et al. 2012; Yakel 2013). Thus, it is expected that inhibitors of acetylcholinesterase activity does not induce reversal of hyperlocomotion induced by ketamine. Our data corroborate with a study performed by Csernansky et al. (2005) which also showed that AChE inhibitor (donepezil and galantamine) did not reversed the hyperlocomotion produced by MK-801, an animal model of schizophrenia performed in mice.

Our results also indicate that rivastigmine was effective to improve the cognitive deficit on different task (immediate memory, short term and long term memory) induced by ketamine. Supporting our study, evidences indicated that AChE inhibitor increase ACh availability at synaptic cleft, consequently, the therapeutic effects of these drugs are related to cholinergic stimulation in the brain structures involved in the regulation of cognitive and behavioral processes (Nieoullon 2010). Ours results are in accordance with findings which suggest that rivastigmine improves the cognitive function in schizophrenic patients (Lenzi et al. 2003; Aasen et al. 2005; Kumari et al. 2006). An interesting meta-analysis of the literature performed Ribeiz et al. (2010) indicated that specific cognitive deficits (memory, and the motor speed and attention part of executive function) of patients with schizophrenia and schizoaffective disorder are responsive to rivastigmine, donepezil and galantamine as adjunctive therapy.

Moreover, the present study showed that chronic ketamine administration increase degradation of ACh in the prefrontal cortex, hippocampus and striatum. These brain structures are closely related to behavior and biochemical alterations in schizophrenia (Tzschentke and Schmidt 2000; Cardinal et al. 2002; Sesack and Carr 2002; Lodge and Grace 2011; Wolf et al. 2011). In this line, a study performed by Karson et al. (1998) showed that cognitive impairment was correlated with reduction of mesopontine choline acetyltransferase (ChAT) activity, analyzed in *postmortem* brain of schizophrenic patients. This enzyme is extensively distributed throughout the CNS where, catalyzes the ACh synthesis (Oda 1999). Moreover, another study indicated that ChAT activity was significantly lower in nucleus accumbens from psychotic patients (Bird et al. 1997). In addition, *postmortem* study carried out by Freedman et al. (2000), has shown a reduction in the density of alpha7 neuronal nicotinic acetylcholine receptors in the hippocampus, striatum and prefrontal cortex of schizophrenic patients. These evidences indicate that schizophrenic patients have cholinergic abnormalities such as reduced acetylcholine in different brain structures (Bencherif et al. 2012). On the other hand, Nelson et al. (2002) showed that ketamine induced increase in cortical ACh release. These results corroborate with a research performed by Chatterjee et al. (2012) which shows that ketamine (acute and chronic) mediated increase in ACh concentration in the cerebral cortex and

ketamine also induced increase in AChE activity in the cerebral cortex and hippocampus. This increase can be associated to a neuronal protection mechanism. Therefore, these findings indicate that activation of AChE in the cortical and hippocampal areas suggest an increased ACh turnover, further lends to idea that the ketamine induces cognitive deficit by cholinergic deregulation.

Another evidence that indicates a change in the cholinergic system in schizophrenic patients is the fact that schizophrenic patients smoke cigarettes at a much higher rate than in the general population (Sagud et al. 2009), since nicotine activates the cholinergic system by nicotinic receptors (Williams and Ganghi 2008; Winterer 2010). Data from literature have shown that consuming nicotine may ameliorate the negative symptoms of schizophrenia, leading to increased social interactions, pleasurable activities and improve of cognitive symptoms (Levin et al. 1996; Beratis et al. 2001; Combs and Advokat 2000; Poirier et al. 2002; DeLuca et al. 2004; Harris et al. 2004; Esterberg and Compton 2005). Therefore, nicotinic drugs may be an important therapeutic target to schizophrenia since that clinical studies indicated improve of attention consequently cognitive improve. However, it is important to observe the dosage and dosing schedule of these drugs. Moreover, a better understanding of the human biology of nicotinic receptor is essential for improving the successful clinical development of this promising target (AhnAllen 2012).

It is important to emphasize that few studies have investigated the cognitive role of rivastigmine in animal model relevant to schizophrenia. A preclinical study showed that AChE inhibitors such as physostigmine and donepezil improved the cognitive deficits induced by MK-801 in mice, an animal model of schizophrenia (Csernansky et al. 2005). Therefore, ours results become relevant to reinforce that AChE inhibitors such as rivastigmine, may be important to lessen the cognitive deficits produced by ketamine, another animal model of schizophrenia. Also, our results point to the need to assess AChE inhibitors in different animal model of schizophrenia since each animal model has different characteristics (specie of rodent, dose, time, effect and duration of effect). Besides the evaluation of behavioral effects of rivastigmine, we also assessed the effect of this AChE inhibitor drug in activity of acetylcholinesterase in the prefrontal cortex, hippocampus and striatum. The results of this work may have relevance for the behavioral and biochemical effects of cholinomimetic drugs in patients with schizophrenia.

In conclusion, this work indicates that rivastigmine might be effective in the reduction of cognitive deficit in different task (immediate memory, short term and long term memory) induced by chronic ketamine, an animal model of schizophrenia in rats. Moreover, rivastigmine reversed the increase of AChE activity in the prefrontal cortex, hippocampus and striatum of rats subjected to ketamine-induced animal model

of schizophrenia. This effect may contribute to improve of cognitive impairment induced by rivastigmine on this animal model. However, this AChE inhibitor did not reverse the positive symptoms of schizophrenia (ketamine-induced hyperlocomotion), indicating specificity on cognitive function in the schizophrenia. Cholinergic system might be an important therapeutic target in the physiopathology of schizophrenia, mainly in the cognition, but additional studies should be carried.

Financial Support This Project was supported by L'Oreal, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Universidade do Extremo Sul Catarinense (UNESC) and FAPESC.

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