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Effect of folic acid on oxidative stress and behavioral changes in the animal model of schizophrenia induced by ketamine

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ABSTRACT

Recent studies have shown benefits for the supplementation of folic acid in schizophrenic patients. The aim of this study was to evaluate the effects of folic acid addition on adult rats, over a period of 7 or 14 days. It also sets out to verify any potential protective action using an animal model of schizophrenia induced by ketamine, in behavioral and biochemical parameters. This study used two protocols (acute and chronic) for the administration of ketamine at a dose of 25 mg/kg (i.p.). The folic acid was given by oral route in doses of 5, 10 and 50 mg/kg, once daily, for 7 and/or 14 days in order to compare the protective effects of folic acid. Thirty minutes after the last administration of ketamine, the locomotor and social interaction activities were evaluated, and immediately the brain structure were removed for biochemical analysis. In this study, ketamine was administered in a single dose or in doses over the course of 7 days increasing the animal's locomotion. This study showed that the administration of folic acid over 7 days was unable to prevent hyper locomotion. In contrast, folic acid (10 and 50 mg/kg) administrated over a period of 14 days, was able to partially prevent the hyper locomotion. Our data indicates that both acute and chronic administrations of ketamine increased the time to first contact between the animals, while the increased latency for social contact was completely prevented by folic acid (5, 10 and 50 mg/kg). Chronic and acute administrations of ketamine also increased lipid peroxidation and protein carbonylation in brain. Folic acid (10 and 50 mg/kg) supplements showed protective effects on the oxidative damage found in the different brain structures evaluated. All together, the results indicate that nutritional supplementation with folic acid provides promising results in an animal model of schizophrenia induced by ketamine.

1. Introduction

Folic acids one of the B complex vitamins which participates in the metabolism of one-carbon (C1) compounds (Coppen and Bolander-Gouaille, 2005; Mattson and Shea, 2003). This vitamin plays a neuro protective role against impairments in the central nervous system (CNS), and promotes neuronal growth and repair (Budni et al., 2011; Iskandar et al., 2004). Folic acid is also involved in the metabolism and functioning of compounds essential to the CNS, which includes the purines, pyrimidines, DNA, RNA, amino acids, phosphate compounds, vitamin B-12, methionine, S-adenosylmethionine, dopamine, adrenaline, noradrenaline and serotonin (Coppen and Bolander-Gouaille, 2005; Kronenberg et al., 2009; Lucock, 2011;). Thus, alterations in the metabolism of folic acid can

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directly affect the CNS (Coppen and Bolander-Gouaille, 2005; Krebs et al., 2009; Miller, 2008; Stahl, 2007).

The presence of neuropsychiatric symptoms in patients with B deficiency vitamins complex and in children conceived following a short inter-pregnancy interval indicates a role for folic acid in schizophrenia. The influence of the interval between pregnancies could be explained by the reduction of the maternal reserves of folic acid when the maternal folic acid stores are still being replenished (Dogan et al., 2009).

Schizophrenia is a severe, chronic and debilitating mental disorder that affects 1% of the World's population, and is characterized by positive (e.g. hallucinations), negative (e.g. blunted affects and social isolation) and cognitive symptoms (e.g. executive and memory dysfunction) (Larson et al., 2010). This disorder is considered to be the result of a genetic combination and environmental factors. Although its pathophysiology has not been fully determined, biological studies support that the involvement of several possible components, including altered DNA methylation, abnormal transmission of neurotransmitters, oxidative stress, folic acid deficiencies and high maternal homocysteine levels. Each of these factors has been separately explored, and they have all been found to involve C1 metabolism, which is a putative target-integrating gene–environment interactions by influencing epigenetic regulation (Krebs et al., 2009). Studies have indicated an important relationship between folic acid metabolism and schizophrenia. In this sense, these findings support the hypothesis that the deficiency of this vitamin during fetal development may be an important risking factor for schizophrenia (Gunawardana et al., 2011).

Current studies utilize pharmacological tools in evaluating the effects of new protective compounds against schizophrenia. Recent reviews have shown that ketamine is a useful tool for studying the positive, negative and cognitive symptoms observed in acute schizophrenia (Frohlich and Van Horn, 2014). This animal model of schizophrenia involves the acute or repeated administration of ketamine (Becker and Grecksch, 2004; Bubeníková-Valesová et al., 2008; Canever et al., 2010; De Oliveira et al., 2011). Ketamine is a dissociative anesthetic which acts as an NMDA receptor non competitive antagonist, and is largely used within research to create animal models of schizophrenia; since it induces schizophrenia-like symptoms (Dingledine et al., 1999). These animal models imitate the behavioral changes seen in schizophrenia such as hyperactivity, social interaction and memory deficits. Moreover, ketamine induces similar biochemical alterations that are found in schizophrenia, such NMDA receptor hypo function and oxidative stress (Chatterjee et al., 2011). Based on these previous results from our laboratory support the hypothesis that early insults interfere with the glutamatergic system, reflecting a greater sensitivity to the effects of ketamine in adulthood within an animal model of schizophrenia (Zugno et al., 2013). All these findings reinforce the validity of the ketamine model since it is capable to mimic the phenotype of schizophrenia in both, the animal behavior as well as in the biochemical alterations seen in brain.

Considering that the etiology of schizophrenia is not clear, current researches indicate that the accumulation of reactive oxygen species (ROS) is associated with the pathophysiology of the disorder (Ciobica et al., 2011; Ruiz-Litago et al., 2012; Yao and Keshavan, 2011). Oxidative stress occurs because of the increased levels of ROS, reactive nitrogen species (RNS) or by an imbalance in the activity of the endogenous antioxidant system (Berg et al., 2004; Kwon et al., 2003). As a consequence of the increased levels of ROS and the failure of the endogenous antioxidant system, damage to DNA, proteins and membrane lipids can occur (Konat, 2003; Kwon et al., 2003).

The link between schizophrenia and oxidative stress was recently demonstrated when it was shown that the activity of glutathione peroxides (GPx) decreased in both treated and untreated patients (Miljevic et al., 2010). Also, increased levels of markers for lipid per oxidation were observed in a similar population (Dadheech et al., 2008; Padurariu et al., 2010). Additionally, a study performed by our group suggested that the animal model of schizophrenia induced by ketamine showed changes in the activity of superoxide dismutase (SOD), catalase (CAT) and GPx, resulting in protein and lipid damage (De Oliveira et al., 2009).

Folic acid is involved in the metabolism of homocysteine that can be remethylated to methionine by enzymes that require folic acid, and it can also be catabolized by cystathionine-B-synthase, an enzyme dependent on the vitamin B6, that forms cysteine, a precursor of glutathione (Micle et al., 2012). It is known that hyperhomocysteinemia is directly related to oxidative stress. The autoxidation of homocysteine and other disulfides, releases oxygen (O_2) and hydrogen peroxide (H_2O_2), both of them impair neuronal function and predispose the neuronal tissue to neurodegenerative and psychiatric disorders (White et al., 2001). Furthermore, the deficiency of folic acid induces the high levels of homocysteine and the formation of ROS that lead to decreases in both antioxidant potential and the activity of GPx, increasing oxidative tissue damage (McCully, 2009).

Although, studies have emphasized the association between folic acid deficiency and schizophrenia, few preclinical studies have been conducted to study the supplementation of this vitamin, or to evaluate its effect in an animal model of schizophrenia (Godfrey et al., 1990; Levine et al., 2006). In this sense, the aim of the study was to evaluate the effect of diet supplementation with folic acid over a period of seven or fourteen days in adult rats, and also to verify potential protective actions in an animal model of schizophrenia induced by acute or chronic administrations of ketamine on the animals behavioral and biochemical parameters.

2. Methods

2.1. Experimental procedures

The animals included in this study were handled according to the NIH Guide for the Care and Usage of Laboratory Animals, and also in accordance with the rules of the Brazilian Society for Neuroscience and Behavior (SBNeC). The experiments were performed at the Universidade do Extremo Sul Catarinense (UNESC) Brazil, in the Laboratory of Neurosciences, and in partnership with the Laboratory of Pathophysiology. All experimental procedures were performed in accordance with international recommendations for the care and use of laboratory animals, and additionally in compliance with the recommendations for the use of animals as set out by the Brazilian Society of Neuroscience and Behavior (SBNeC).

2.2. Animals

Sixty day old male *Wistar* rats weighing around 250–300 g were obtained from our breeding colony. They were kept in cages (41 × 34 cm and 16 cm high) 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 a.m.), at a temperature of 23 ± 1 °C. These conditions were maintained constantly throughout the experiments. Our study used 80 animals for each experimental protocol (7 and 14 days) (n = 10) animals per group, consisting of 160 animals in the study. All experimental procedures were performed in accordance with, and with the approval of, the local Ethics Committee for the Use of Animals (Protocol 49/2012). All efforts were made to minimize animal suffering and to reduce the number of animals used in this study, utilizing alternatives to in vivo techniques when