Effect of acute administration of ketamine and imipramine on Creatine kinase activity in the brain of rats
Efeito da administração aguda da cetamina e imipramina sobre a atividade da creatina quinase no encéfalo de ratos

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Abstract
Objective: Clinical findings suggest that ketamine may be used for the treatment of major depression. The present study aimed to compare behavioral effects and brain Creatine kinase activity in specific brain regions after administration of ketamine and imipramine in rats.
Method: Rats were acutely given ketamine or imipramine and antidepressant-like activity was assessed by the forced swimming test; Creatine kinase activity was measured in different regions of the brain.
Results: The results showed that ketamine (10 and 15mg/kg) and imipramine (20 and 30mg/kg) reduced immobility time when compared to saline group. We also observed that ketamine (10 and 15mg/kg) and imipramine (20 and 30mg/kg) increased Creatine kinase activity in striatum and cerebral cortex. Ketamine at the highest dose (15mg/kg) and imipramine (20 and 30mg/kg) increased Creatine kinase activity in cerebellum and prefrontal cortex. On the other hand, hippocampus was not affected.
Conclusion: Considering that metabolism impairment is probably involved in the pathophysiology of depressive disorders, the modulation of energy metabolism (like increase in Creatine kinase activity) by antidepressants could be an important mechanism of action of these drugs.

Descriptors: Imipramine; Creatine kinase; Depression; Brain; Rats

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Introduction
Depressive disorders, including major depression, are serious and disabling. It is thought that one in every five individual will suffer from a mood disorder in their lifetime. Pharmacotherapy of depression is costly and widely prescribed by physicians; even though less than half of the patients treated attain complete remission after therapy with a single antidepressant. Others exhibit partial, refractory or intolerant responses to pharmacological treatment, emphasizing the need to discover novel antidepressants. New refractory or intolerant responses to pharmacological treatment, after therapy with a single antidepressant. Others exhibit partial, though less than half of the patients treated attain complete remission of antidepressant response, broader efficacy, and fewer adverse effects. In this context, it is extremely important to develop potent treatments with quick action for major depression.

A growing body of evidence has pointed to the ionotropic glutamate N-methyl-D-aspartate receptor (NMDA) as an important player in the etiology of psychopathologies, including anxiety and major depression. Several clinical and preclinical studies have demonstrated that NMDA antagonists produce anxiolytic- and antidepressant-like effects. In this way, chemical compounds acting on NMDA receptors could be interesting as pharmacological targets for the treatment of mood disorders.

Ketamine is a non-competitive antagonist to the phencyclidine site of NMDA glutamate receptor, which works in a use- and voltage-dependent manner. Clinical studies suggested that acute administration of ketamine ameliorate depressive symptoms in patients suffering from major depression. Moreover, preclinical studies also demonstrated that ketamine induces anxiolytic- and antidepressant-like effects in rodents subjected to animal models of anxiety and depression. In agreement with these findings, we have also recently demonstrated that acute and chronic administration of ketamine decreased immobility time in the forced swimming test. Correll and Futter observed a mild feeling of “headness” or inebriation as the only adverse effect of ketamine used in dose well below the anesthetic dose. No sedation or hallucinations or changes in liver function were observed, and there were no changes in blood pressure or pulse.

Creatine kinase (CK; E.C. 2.7.3.2) plays a central role in metabolism of high-energy consuming tissues such as brain, where it functions as an effective buffering system of cellular adenosine triphosphate (ATP) levels. The enzyme catalyzes the reversible transfer of the phosphoryl group from phosphocreatine to adenosine diphosphate (ADP), regenerating ATP. It is believed that during excitation a 10-fold increase of cellular turnover occurs, and that during these rapids changes the Creatine/phosphocreatine/Creatine kinase system is necessary as an energy buffering system to avoid large fluctuations of cellular ATP/ADP levels in excitable tissues.

It is also known that a decrease in Creatine kinase activity may potentially impair energy homeostasis, contributing to cell death. We have recently showed that brain CK activity is inhibited by antipsychotics (haloperidol and olanzapine), in animal models of neuropsychiatry disorders, such as bipolar disorder and after electroconvulsive shock.

Brain and other high-energy consuming tissues are more susceptible to reduction of energy metabolism. Neuropsychiatric disorders, such as schizophrenia, depression and bipolar disorder have been related to dysfunction in brain metabolism. In this context, the main objective of our study was to compare behavioral and biochemical effects induced by acute administration of ketamine or imipramine in rats (imipramine was used as a positive control, as a very well known antidepressant). The behavioral effects of both drugs were evaluated in the forced swimming test, which is a behavioral despair assay widely used for screening antidepressant drugs. Moreover, CK activity was evaluated in brain of rats submitted to acute administration of ketamine or imipramine.

Method
1. Animals
Male adult Wistar rats (60 days old, 250-300g) were obtained from Universidade do Estado Sul Catarinense (UNESC, Criciúma, Brazil) breeding colony. Four were housed per cage with food and water available ad libitum and maintained on a 12-h light/ dark cycle (lights on at 7:00 AM). All experimental procedures involving animals were performed following the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNer) recommendations for animal care, with the approval of UNESC Ethics Committee of accord with protocol number 543/2007. Additionally, all efforts were made to minimize animal suffering as well as to reduce the number of animals.

2. Acute administration of ketamine and imipramine
Ketamine was obtained from Fort Dodge (Brazil) and imipramine, the standard antidepressant, from Novartis Pharmaceutical Industry (Brazil). Different groups of rats (n = 8 each) were administered intraperitoneally with saline or different doses of ketamine (5, 10 and 15mg/kg) or imipramine (10, 20 and 30mg/kg) 60 minutes before the test session (forced swimming test). The range of doses of ketamine employed in this work was chosen based on a previous study, which reported an increase in spontaneous locomotor activity at 25mg/kg, while no changes were observed at 10mg/kg. None of the rats was excluded.

3. Forced swimming test
The forced swimming test was conducted according to previous reports. The test involves two individual exposures to a cylindrical tank with water in which rats cannot touch the bottom of the tank or escape. The tank is made of clear Plexiglas, 80cm tall, 30cm in diameter, and filled with water (22-23°C) to a depth of 40cm. Water in the tank was changed after each rat swimming test section. For the first exposure, rats without drug treatment were placed in the water for 15 min (pre-test session). Twenty-four hours later, rats were placed in the water again for a 5min session (test session), and the immobility time of rats was recorded in seconds. Rats were treated with ketamine, imipramine or saline only 60 min before the second exposure to the cylindrical tank of water (test session).

4. Tissue and homogenate preparation
Immediately after forced swimming test, the rats were killed by decapitation, the brain was removed and cerebellum, striatum, cerebral cortex, prefrontal cortex and hippocampus were collected and homogenized (1:10, w/v) in SETH buffer, pH 7.4 (250mM sucrose, 2mM EDTA, 10mM Trizma base, 50IU/ml heparin). The homogenates were centrifuged at 800 x g for 10 min and the supernatants kept at -70°C until used for CK activity determination. The maximal period between homogenate preparation and enzyme analysis was always less than five days. Protein content was determined by the method described by Lowry et al, using bovine serum albumin as standard.

5. Creatine kinase (CK) activity assay
CK activity was measured in brain homogenates pre-treated with 0.625mM lauryl maltoside. The reaction mixture consisted of...
60mM Tris–HCl, pH 7.5, containing 7mM phosphocreatine, 9mM MgSO₄, and approximately 0.4–1.2µg protein in a final volume of 100µL. After 15 min of pre-incubation at 37°C, the reaction was started by the addition of 0.3µmol of ADP plus 0.08µmol of reduced glutathione. The reaction was stopped after 10min by the addition of 1µmol of p-hydroxymercuribenzoate acid. The Creatine formed was estimated according to the colorimetric method of Hughes. The color was developed by the addition of 100µL 2% α-naphthol and 100µL 0.05% diacetyl in a final volume of 1mL and read spectrophotometrically after 20min at 540nm. Results were expressed as units/min x mg protein.

6. Statistical analysis

To compare the experimental groups, data were analyzed by the one-way analysis of variance. Tukey test for multiple comparisons was used when F was significant. All analyses were performed using the Statistical Package for the Social Science (SPSS) software version 16.0. Critical significance level used for all comparisons was 5%.

Results

In the present work, we evaluated the immobility time in the forced swimming test, which is a behavioral test widely used for screening antidepressant drugs, and CK activity in brain of rats after acute ketamine or imipramine administration. Figure 1 shows that acute administration of imipramine (20 and 30mg/kg) reduced the immobility time of rats when compared to saline (F (6,48) = 7.12; p < 0.05). Acute administration of ketamine (10 and 15mg/kg) also reduced immobility time (F(6,48) = 5.45; p < 0.05).

We also verified that acute administration of ketamine (10 and 15mg/kg) and imipramine (20 and 30mg/kg) increased CK activity in striatum (F(6,48) = 4.89; p < 0.05) (Figure 2) and cerebral cortex (F(6,48) = 6.02; p < 0.05) (Figure 3). Ketamine at the highest dose (15mg/kg) and imipramine (20 and 30mg/kg) also increased CK activity in cerebellum (F(6,48) = 4.76; p < 0.05) (Figure 4) and prefrontal cortex (F(6,48) = 7.11; p < 0.05) (Figure 5). On the other hand, CK activity in hippocampus was not affected by acute administration of ketamine and imipramine (F(6,48) = 0.47; p = 0.42) (Figure 6).

Discussion

The behavioral effects induced by ketamine found in the present study are in agreement with previous reports, which support an
antidepressant action for this drug. New agents for the treatment of depression must present a rapid onset of antidepressant response, broader efficacy, and fewer adverse effects. Kos et al. showed that ketamine produced anti-immobility effects in the mouse tail suspension test, suggesting an antidepressant-like action in mice. Chaturvedi et al. also demonstrated that ketamine reversed the increase in immobility time induced by shock in the mouse forced swimming test. Finally, Yilmaz et al. showed that one single injection of ketamine (160mg/kg, an anesthetic dose) induced antidepressant-like effects in rats tested in 3, 7, or 10 days after the forced swimming test. Taking together previous reports and our present findings, the antidepressant-like effects induced by ketamine administration in animals are supported (in a dose-dependent manner).

Some interesting evidence showing antidepressant effects of ketamine in humans were also reported. Berman et al. and Zarate et al. suggest that ketamine induce robust and rapid antidepressant effects in depressed patients after a single intravenous injection. In the present work, we also demonstrated the effect of ketamine and imipramine on CK activity in brain of rats. We verified that acute administration of both drugs increased CK activity in striatum, cerebral cortex, cerebellum and prefrontal cortex. CK activity in hippocampus was not affected by ketamine and imipramine. The reason for the lack of effect in hippocampus is not known. CK is an important enzyme responsible for normal energy homeostasis performing several integrated functions, such as temporary energy buffering, metabolic capacity, energy transfer and metabolic control. In this context, the brain presents high levels of phosphocreatine and CK activity. It is well described that the inhibition of CK activity has been implicated in the pathogenesis of a number of diseases, especially in the brain.

Several studies link brain energy metabolism impairment to neuronal death and neurodegeneration. Damage to the mitochondrial electron transport chain has been suggested to be an important factor in the pathogenesis of a range of psychiatric disorders including major depression. Gardner et al. showed a significant decrease in mitochondrial ATP production rates and mitochondrial enzyme ratios in muscles of major depressive disorder patients. Madrigal et al. also reported that complexes I-III and II-III of mitochondrial respiratory chain were inhibited in rat brain after chronic stress (immobilization for six hours during 21 days). We have also recently demonstrated that mitochondrial respiratory chain is inhibited in brain of rats after chronic variable stress (40 days), suggesting that energy metabolism impairment may occur in depressive disorders. Considering that metabolism impairment is probably involved in the pathophysiology of depressive disorders, the modulation of energy metabolism (like increase in CK activity) by antidepressants could be an important mechanism of action of these drugs. Further studies are currently being carried out in order to evaluate whether other enzymes involved in metabolism are also affected by antidepressants, especially ketamine. It is also important to study the effect of other antidepressants on CK activity, in order to determine whether the modulation of this enzyme is one of the mechanisms of action of these drugs.

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