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

Resumo
Objetivo: Vários achados clínicos sugerem que a cetamina apresenta efeito antidepressivo. O presente estudo tem como objetivo comparar efeitos comportamentais e a atividade da creatina quinase em regiões específicas do encéfalo após a administração de cetamina e imipramina em ratos. Método: Ratos Wistar receberam uma administração aguda de cetamina ou imipramina e a atividade antidepressiva foi avaliada pelo teste de nado forçado; a atividade da creatina quinase foi medida em diferentes regiões encefálicas. Resultados: Os resultados mostraram que a cetamina (10 e 15mg/kg) e a imipramina (20 e 30mg/kg) diminuíram o tempo de imobilidade quando comparados ao grupo salina. Também foi observado que a cetamina (10 e 15mg/kg) e a imipramina (20 e 30mg/kg) aumentaram a atividade da creatina quinase no estriado e córtex cerebral. A dose mais alta de cetamina (15mg/kg) e a imipramina (20 e 30mg/kg) aumentaram a atividade da creatina quinase no cerebelo e córtex pré-frontal. Por outro lado, o hipocampo não foi alterado. Conclusão: Considerando que a diminuição no metabolismo provavelmente está envolvida na fisiopatologia da depressão, a modulação do metabolismo energético (como um aumento na atividade da creatina quinase) por antidepressivos pode ser um importante mecanismo de ação destes fármacos.
Descritores: Imipramina; Creatina quinase; Depressão; Encefalo; Ratos

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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
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of depression is costly and widely prescribed by physicians; even
antipsychotics (haloperidol and olanzapine), in animal models
potentially impair energy homeostasis, contributing to cell death.27-
both drugs were evaluated in the forced swimming test, which is a
as a very well known antidepressant). The behavioral effects of
or imipramine in rats (imipramine was used as a positive control,
and biochemical effects induced by acute administration of ketamine
context, the main objective of our study was to compare behavioral
effects.3,4 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.2,3 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.7,15

Ketamine is a non-competitive antagonist to the phencyclidine
site of NMDA glutamate receptor, which works in a use- and
voltage-dependent manner.26 Clinical studies suggested that acute
administration of ketamine ameliorate depressive symptoms in
patients suffering from major depression.4,17 Moreover, preclinical
studies also demonstrated that ketamine induces anxiolytic- and
antidepressant-like effects in rodents subjected to animal models
of anxiety and depression.18-21 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.22 Correll and Futter observed a mild feeling of
“headiness” or inebriation as the only adverse effect of ketamine
used in dose well below the anesthetic dose.23 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 rapid 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.24
It is also known that a decrease in Creatine kinase activity may
potentially impair energy homeostasis, contributing to cell death.27

We have recently showed that brain CK activity is inhibited by
antipsychotics (haloperidol and olanzapine),25 in animal models
of neuropsychiatry disorders, such as bipolar disorder26 and after
electroconvulsive shock.27

Brain and other high-energy consuming tissues are more
susceptible to reduction of energy metabolism. Neuropsychiatry
disorders, such as schizophrenia, depression and bipolar disorder
have been related to dysfunction in brain metabolism.26,28 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.37 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 Extremo 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 (SBNeC) 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.38 None
of the rats was excluded.

3. Forced swimming test
The forced swimming test was conducted according to previous
reports.39,40 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 Trisbase, 50mM lactate/milk). 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.41 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

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Figure 1 – Effects of the acute administration of ketamine (5, 10 and 15mg/kg, i.p.) and imipramine (10, 20 and 30mg/kg, i.p.) on the immobility time of rats subjected to the forced swimming test. Data are expressed as mean ± S.D. (n = 8).
* p < 0.05 vs. saline according to ANOVA followed by Tukey.

Figure 2 – Effects of the acute administration of ketamine (5, 10 and 15mg/kg, i.p.) and imipramine (10, 20 and 30mg/kg, i.p.) on Creatine kinase activity in striatum of rats. Data are expressed as mean ± S.D. (n = 8).
* p < 0.05 vs. saline according to ANOVA followed by Tukey.

Figure 3 – Effects of the acute administration of ketamine (5, 10 and 15mg/kg, i.p.) and imipramine (10, 20 and 30mg/kg, i.p.) on Creatine kinase activity in cerebral cortex
* p < 0.05 vs. saline according to ANOVA followed by Tukey.

Figure 4 – Effects of the acute administration of ketamine (5, 10 and 15mg/kg, i.p.) and imipramine (10, 20 and 30mg/kg, i.p.) on Creatine kinase activity in cerebellum of rats. Data are expressed as mean ± S.D. (n = 8).
* p < 0.05 vs. saline according to ANOVA followed by Tukey.
Ketamine, imipramine and Creatine kinase


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. showed that one single dose of ketamine rapidly improved depressive symptoms in patients with major depression. 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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Disclosures

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* Modest
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Note: UNESCO = Universidade do Extremo Sul Catarinense.

For more information, see instructions for authors.

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