ORIGINAL ARTICLE

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; Encéfalo; 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.[1] 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.[2] New agents for the treatment of depression should present a rapid onset of antidepressant response, broader efficacy, and fewer adverse 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.[5,6] 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.[16] 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 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.[24-26] It is also known that a decrease in Creatine kinase activity may potentially impair energy homeostasis, contributing to cell death.[27-31] We have recently showed that brain CK activity is inhibited by antipsychotics (haloperidol and olanzapine),[32] in animal models of neuropsychiatry disorders, such as bipolar disorder[33] and after electroconvulsive shock.[34]

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.[35,36] 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[33,34]) 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 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.[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 $MgSO_4$, 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.[42] The color was developed by the addition of 100μL 2% α-naphtol 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,[36] 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

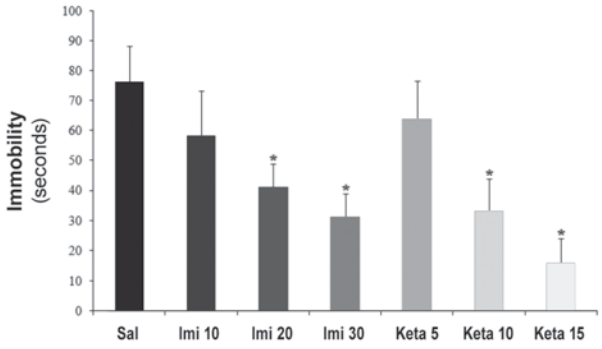

Figure 1 – Effects of the acute administration of ketamine (5, 10 and 15mg/kg, i.p.) and imipramine (10, 20 and 30mg/kg, i.p.) on the immobility time of rats subjected to the forced swimming test. Data are expressed as mean ± S.D. (n = 8).
** p < 0.05 vs. saline according to ANOVA followed by Tukey.*

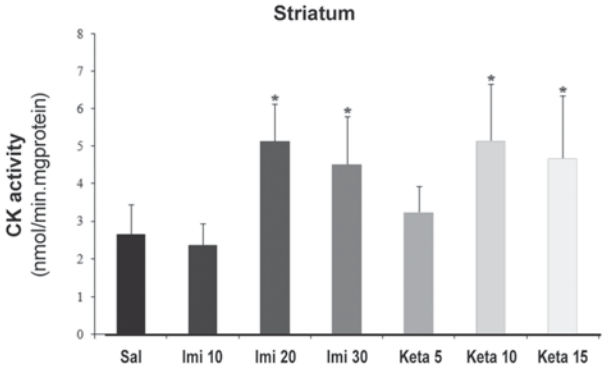

Figure 2 – Effects of the acute administration of ketamine (5, 10 and 15mg/kg, i.p.) and imipramine (10, 20 and 30mg/kg, i.p.) on Creatine kinase activity in striatum of rats. Data are expressed as mean ± S.D. (n = 8).
** p < 0.05 vs. saline according to ANOVA followed by Tukey.*

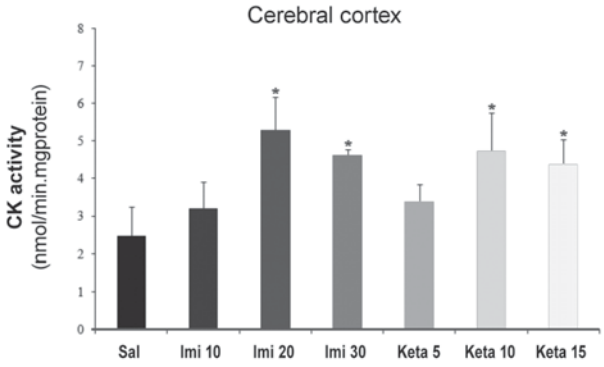

Figure 3 – Effects of the acute administration of ketamine (5, 10 and 15mg/kg, i.p.) and imipramine (10, 20 and 30mg/kg, i.p.) on Creatine kinase activity in cerebral cortex
** p < 0.05 vs. saline according to ANOVA followed by Tukey.*

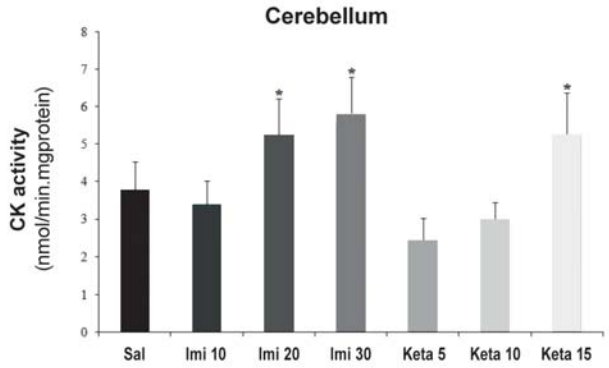

Figure 4 – Effects of the acute administration of ketamine (5, 10 and 15mg/kg, i.p.) and imipramine (10, 20 and 30mg/kg, i.p.) on Creatine kinase activity in cerebellum of rats. Data are expressed as mean ± S.D. (n = 8).
** p < 0.05 vs. saline according to ANOVA followed by Tukey.*

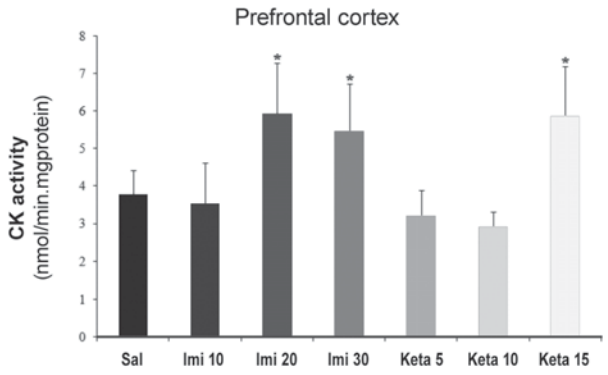

Figure 5 – Effects of the acute administration of ketamine (5, 10 and 15mg/kg, i.p.) and imipramine (10, 20 and 30mg/kg, i.p.) on Creatine kinase activity in prefrontal cortex of rats. Data are expressed as mean ± S.D. (n = 8).
** p < 0.05 vs. saline according to ANOVA followed by Tukey.*

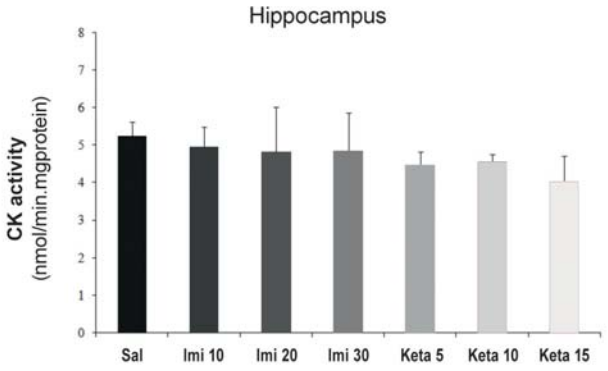

Figure 6 – Effects of the acute administration of ketamine (5, 10 and 15mg/kg, i.p.) and imipramine (10, 20 and 30mg/kg, i.p.) on Creatine kinase activity in hippocampus of rats. Data are expressed as mean ± S.D. (n = 8).

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.[3,4] Kos et al. showed that ketamine produced anti-immobility effects in the mouse tail suspension test, suggesting an antidepressant-like action in mice.[21] Chaturvedi et al. also demonstrated that ketamine reversed the increase in immobility time induced by shock in the mouse forced swimming test.[19] 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.[20] 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.[4,17] Zarate et al. suggest that ketamine induce robust and rapid antidepressant effects in depressed patients after a single intravenous injection.[4]

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.[43,44]

Several studies link brain energy metabolism impairment to neuronal death and neurodegeneration.[45-47] Damage to the mitochondrial electron transport chain has been suggested to be an important factor in the pathogenesis of a range of psychiatric disorders[33,48-51] 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.[52] 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).[48] 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.[51] 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

Writting group member	Employment	Research grant[1]	Other research grant or medical continuous education[2]	Speaker's honoraria	Ownership interest	Consultant/ Advisory board	Other[3]
Lara C. Assis	UNESC	-	-	-	-	-	-
Gislaine T. Rezin	UNESC	-	-	-	-	-	-
Clarissa M. Comim	UNESC	-	-	-	-	-	-
Samira S. Valvassori	UNESC	-	-	-	-	-	-
Isabela C. Jeremias	UNESC	-	-	-	-	-	-
Alexandra I. Zugno	UNESC	-	-	-	-	-	-
João Quevedo	UNESC	-	-	-	-	-	-
Emilio L. Streck	UNESC	-	-	-	-	-	-

** Modest*
*** Significant*
**** Significant. Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.*
Note: UNESC = Universidade do Extremo Sul Catarinense.
For more information, see Instructions for authors.

References

1. 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(92):303-7.
2. Pacher P, Kohegyi E, Kecskemeti V, Furst S. Current trends in the development of new antidepressants. *Curr Med Chem.* 2001;8(2):89-100.
3. Nutt DJ. The neuropharmacology of serotonin and noradrenaline in depression. *Int Clin Psychopharmacol.* 2002;17(Suppl 1):S1-12.
4. Zarate CA Jr, Singh JB, Carson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, Charney DS, Manji HK. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry.* 2006;63(8):856-64.
5. Krystal JH, D'Souza DC, Petrakis IL, Belger A, Berman RM, Charney DS, Abi-Saab W, Madonick S. NMDA agonists and antagonists as probes of glutamatergic dysfunction and pharmacotherapies in neuropsychiatric disorders. *Harv Rev Psychiatry.* 1999;7(3): 125-43.
6. Javitt DC. Glutamate as a therapeutic target in psychiatric disorders. *Mol Psychiatry.* 2004;9(11):984-79.
7. Trullas R, Skolnick P. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur J Pharmacol.* 1990;185(1):1-10.
8. Maj J, Rogoz Z, Skuza G, Sowińska H. The effect of CGP 37849 and CGP 39551, competitive NMDA receptor antagonists, in the forced swimming test. *Pol J Pharmacol Pharm.* 1992;44(4):337-46.
9. Skolnick P, Layer RT, Popik P, Nowak G, Paul IA, Trullas R. Adaptation of N-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: implications for the pharmacotherapy of depression. *Pharmacopsychiatry.* 1996;29(1):23-6.
10. Matheus MG, Guimarães FS. Antagonism of non-NMDA receptors in the dorsal periaqueductal grey induces anxiolytic effect in the elevated plus maze. *Psychopharmacology* (Berl). 1997;132(1):14-8.
11. Przegalinski E, Tatarczynska E, Deren-Wesolek A, Chojnacka-Wojcik E. Antidepressant-like effects of a partial agonist at strychnine-insensitive glycine receptors and a competitive NMDA receptor antagonist. *Neuropharmacology.* 1997;36(1):31-7.
12. Adamec RE, Burton P, Shallow T, Budgell J. Unilateral block of lasting increases in anxiety-like behavior and unconditioned startle effective hemisphere depends on the behavior. *Physiol Behav.* 1999;65(4-5):739-51.
13. Menard J, Treit D. Intra-septal infusions of excitatory amino acid receptor antagonists have differential effects in two animal models of anxiety. *Behav Pharmacol.* 2000;11(2):99-108.
14. Molchanov ML, Guimarães FS. Anxiolytic-like effects of AP7 injected into the dorsolateral or ventrolateral columns of the periaqueductal gray of rats. *Psychopharmacology* (Berl*).* 2002;160(1):30-8.
15. Kos T, Legutko B, Danysz W, Samoriski G, Popik P. Enhancement of antidepressant-like effects but not brain-derived neurotrophic factor mRNA expression by the novel N-methyl-D-aspartate receptor antagonist neramexane in mice. *J Pharmacol Exp Ther.* 2006a;318(3):1128-36.
16. Hirota K, Lambert DJ. Ketamine: its mechanism(s) of action and unusual clinical uses. *Br J Anaesth.* 1996;77(4):441-4.
17. Berman RB, Cappielo A, Anand A, Oren DA, Heninger GR, Charney DS, Krystal JH. Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry.* 2000;47(4):351-4.
18. Silvestre JS, Nadal R, Pallares M, Ferré N. Acute effects of ketamine in the holeboard, the elevated-plus maze, and the social interaction test in Wistar rats. *Depress Anxiety.* 1997;5(1):29-33.
19. Chaturvedi HK, Bapna JS, Chandra D. Effect of fluvoxamine and N-methyl-D-aspartate receptor antagonists on shock-induced depression in mice. *Indian J Physiol Pharmacol.* 2001;45(2):199-207.
20. Yilmaz A, Schulz D, Aksoy A, Canbeyli R. Prolonged effect of an anesthetic dose of ketamine on behavioral despair. *Pharmacol Biochem Behav.* 2002;71(1-2):349-52.
21. Kos T, Popik P, Pietraszek M, Schäfer D, Danysz W, Dravolina O, Blokhina E, Galankin T, Bespalov AY. Effect of 5-HT3 receptor antagonist MDL 72222 on behaviors induced by ketamine in rats and mice. *Eur Neuropsychopharmacol.* 2006b;16(4):297-310.
22. Garcia LS, Comim CM, Valvassori SS, Réus GZ, Barbosa LM, Andreazza AC, Stertz L, Fries GR, Gavioli EC, Kapczinski F, Quevedo J. Acute administration of ketamine induces antidepressant-like effects in the forced swimming test and increases BDNF levels in the rat hippocampus. *Prog Neuropsychopharmacol Biol Psychiatry.* 2008;32(1):140-4.
23. Correl GE, Futter GE. Two case studies of patients with major depressive disorder given low-dose (subanesthetic) ketamine infusions. *Pain Med.* 2006;7(1):92-5.
24. Bessman SP, Carpenter CL. The Creatine-Creatine phosphate energy shuttle. *Annu Rev Biochem.* 1985;54:831-65.
25. Schnyder T, Gross H, Winkler H, Eppenberger HM, Wallimann T. Crystallization of mitochondrial Creatine kinase. Growing of large protein crystals and electron microscopic investigation of microcrystals consisting of octamers. *J Biol Chem.* 1991;266(8):5318-22.
26. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM. Intracellular compartmentation, structure and function of Creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphoCreatine circuit' for cellular energy homeostasis. *Biochem J.* 1992;281(PT 1):21-40.
27. 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(5):447-55.
28. Hamman BL, Bittl JA, Jacobus WE, Allen PD, Spencer RS, Tian R, Ingwall JS. Inhibition of the Creatine kinase reaction decreases the contractile reserve of isolated rat hearts. *Am J Physiol.* 1995;269(3 Pt 2):1030-6.
29. Gross WL, Bak MI, Ingwall JS, Arstall MA, Smith TW, Balligand JL, Kelly RA. Nitric oxide inhibits Creatine kinase and regulates rat heart contractile reserve. *Proc Natl Acad Sci USA.* 1996;93(11):5604-9.
30. 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. *Mol Brain Res.* 1998;54(2):276-87.

31. Aksenov M, Aksenov M, Butterfield DA, Markesbery WR. Oxidative modification of Creatine kinase BB in Alzheimer's disease brain. *J Neurochem.* 2000;74(6):2520-7.
32. Assis LC, Scaini G, Di-Pietro PB Castro AA, Comim CM, Streck EL, Quevedo J. Effect of antipsychotics on Creatine kinase activity in rat brain. *Basic Clin Pharmacol Toxicol.* 2007;101(5):315-9.
33. Streck EL, Amboni G, Scaini G, Di-Pietro PB, Rezin GT, Valvassori SS, Luz G, Kapczinski F, Quevedo J. Brain Creatine kinase activity in an animal model of mania. *Life Sci.* 2008;82(7-8):424-9.
34. Burigo M, Roza CA, Bassani C, Feier G, Dal-Pizzol F, Quevedo J, Streck EL. Decreased Creatine kinase activity caused by electroconvulsive shock. *Neurochem Res.* 2006;31(7):877-81.
35. Albert KA, Hemmings HC, Adamo AI, Potkin SG, Akbarian S, Sandman CA, Cotman CW, Bunney WE Jr, Greengard P. Evidence for decreased DARPP-32 in the prefrontal cortex of patients with schizophrenia. *Arch Gen Psychiatry.* 2002;59(8):705-12.
36. Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT, Griffin JL, Wayland M, Freeman T, Dudbridge F, Lilley KS, Karp NA, Hester S, Tkachev D, Mimmack ML, Yolken RH, Webster MJ, Torrey EF, Bahn S. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol Psychiatry.* 2004;9(7):684-97.
37. McArthur R, Borsini F. Animal models of depression in drug discovery: a historical perspective. *Pharmacol Biochem Behav.* 2006;84(3):436-52.
38. Hunt MJ, Rainaud B, Garcia R. Ketamine dose-dependently induces highfrequency oscillations in the nucleus accumbens freely moving rats. *Biol Psychiatry.* 2006;60(11):1206-14.
39. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature.* 1977;266(5604):730-2.
40. Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology* (Berl). 1995;121(1):66-72.
41. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193(1):265-7.
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;7:597-603.
43. Khuchua ZA, Qin W, Boero J, Cheng J, Payne RM, Saks VA, Strauss AW. Octamer formation and coupling of cardiac sarcomeric mitochondrial Creatine kinase are mediated by charged N-terminal residues. *J Biol Chem.* 1998;273(36):22990-6.
44. Schlattner U, Wallimann T. Octamers of mitochondrial Creatine kinase isoenzymes differ in stability and membrane binding. *J Biol Chem.* 2000;275(23):17314-20.
45. Heales SJ, Bolaños JP, Stewart VC, Brookes PS, Land JM, Clark JB. Nitric oxide, mitochondria and neurological disease. *Biochim Biophys Acta.* 1999;1410(2):215-28.
46. Blass JP. Brain metabolism and brain disease: is metabolic deficiency the proximate cause of Alzheimer dementia? *J Neurosci Res. 2001*;66(5):851-6.
47. Schurr A. Energy metabolism, stress hormones and neural recovery from cerebral ischemia/hypoxia. *Neurochem Int.* 2002; 41(1):1-8.
48. Madrigal JL, Olivenza R, Moro MA, Lizasoain I, Lorenzo P, Rodrigo J, Leza JC. Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain. *Neuropsychopharmacology.* 2001;24(4):420-9.
49. Fattal O, Budur K, Vaughan AJ, Franco K. Review of the literature on major mental disorders in adult patients with mitochondrial diseases. *Psychosomatics.* 2006;47(1):1-7.
50. Corrêa C, Amboni G, Assis LC, Martins MR, Kapczinski F, Streck EL, Quevedo J. Effects of lithium and valproate on hippocampus citrate synthase activity in an animal model of mania. *Prog Neuropsychopharmacol Biol Psychiatry.* 2007;31(4):887-91.
51. Rezin GT, Cardoso MR, Gonçalves CL, Scaini G, Fraga DB, Riegel RE, Comim CM, Quevedo J, Streck EL. Inhibition of mitochondrial respiratory chain in brain of rats subjected to an experimental model of depression. *Neurochem Int.* 2008;53(6-8):395-400.
52. Gardner A, Johansson A, Wibom R, Nennesmo I, von Döbeln U, Hagenfeldt L, Hällström T. Alterations of mitochondrial function and correlations with personality traits in selected major depressive disorder patients. *J Affect Dis.* 2003;76(1-3):55-68.