ORIGINAL ARTICLE

Ketamine alters behavior and decreases BDNF levels in the rat brain as a function of time after drug administration

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Objective: To evaluate behavioral changes and brain-derived neurotrophic factor (BDNF) levels in rats subjected to ketamine administration (25 mg/kg) for 7 days.

Method: Behavioral evaluation was undertaken at 1 and 6 hours after the last injection.

Results: We observed hyperlocomotion 1 hour after the last injection and a decrease in locomotion after 6 hours. Immobility time was decreased and climbing time was increased 6 hours after the last injection. BDNF levels were decreased in the prefrontal cortex and amygdala when rats were killed 6 hours after the last injection, compared to the saline group and to rats killed 1 hour after the last injection. BDNF levels in the striatum were decreased in rats killed 6 hours after the last ketamine injection, and BDNF levels in the hippocampus were decreased in the groups that were killed 1 and 6 hours after the last injection.

Conclusion: These results suggest that the effects of ketamine on behavior and BDNF levels are related to the time at which they were evaluated after administration of the drug.

Keywords: Brain-derived-neurotrophic factor; forced swimming test; behavioral; ketamine

Introduction

A growing body of evidence points to involvement of N-methyl-D-aspartate (NMDA) in the pathophysiology and treatment of psychiatric disorders.1,2 In fact, clinical studies indicate that administration of subanesthetic doses of ketamine, an NMDA receptor antagonist, to healthy individuals can mimic the positive and negative symptoms seen in schizophrenic patients,3,4 including social withdrawal and cognitive deficits.5 On the other hand, acute administration of ketamine rapidly improved depressive symptoms in patients with major depression.6

However, demonstrations of the psychotherapeutic effects of ketamine on animal models are complicated by its side effects on general activity, which have not been routinely measured or taken into account in experimental studies.7 Interestingly, glutamate is known to regulate neurogenesis, synaptogenesis, and neuron survival.8 Ketamine plays important roles in synaptic plasticity and neuronal learning that can regulate cell proliferation and dysregulation, which are widely discussed as pathogenetic factors of neuropsychiatric disorders.9

It is well known that neuropsychiatric diseases are associated with alterations in nervous signal transmission or disturbances in growth factors in the brain.10 Brain-derived neurotrophic factor (BDNF) is one of the major neurotrophic factors that primarily support the growth and survival of cholinergic, dopaminergic, and motor neurons. BDNF is synthesized by sensory neurons and glia and may have both autocrine and paracrine functions in mediating activity-dependent plasticity.11 Although the mechanism of NMDA receptor blockade-mediated apoptotic neurodegeneration in the brain is not clearly defined, conflicting reports demonstrate both increases and decreases in BDNF levels after exposure to the non-competitive NMDA receptor antagonist MK-801. A decrease in plasma levels of BDNF has been shown in animal models of schizophrenia,12 and decreased levels in the dorsolateral prefrontal cortex have been reported in individuals with schizophrenia.13 In contrast, other studies have demonstrated an increase in BDNF levels in the cortical areas.14 In addition, reduced levels of BDNF have been reported in the serum, plasma, and brain tissues of animals and humans with depression, and antidepressant treatment has been shown to increase BDNF.15 These contradictory observations may account for both the neuroprotective and proapoptotic nature of MK-801.16

Although there are many studies linking ketamine with neuropsychiatric diseases, behavioral parameters and BDNF levels at different times after the administration of ketamine have yet to be fully characterized. The main objective of this study was therefore to evaluate the
effects of ketamine on the open-field and forced swimming tests at different times after the administration of the drug, as well as to analyze BDNF protein levels in specific rat brain areas (prefrontal cortex, hippocampus, amygdala, and striatum).

**Methods**

**Animals**

Adult male Wistar rats (60 days old) were obtained from the Central Animal Facility of Universidade do Extremo Sul Catarinense (UNESC), Criciúma, state of Santa Catarina, Brazil. They were caged in groups of five with free access to food and water and were maintained on a 12-hour light-dark cycle (lights on 7:00 a.m.), at a temperature of 22 ± 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 the UNESC Ethics Committee.

**Drugs and treatment**

A chronic subanesthetic dose (25 mg/kg) of ketamine (Fort Dodge, Brazil) was administered once a day over 7 consecutive days, intraperitoneally (i.p.), at a volume of 1 mL/100 g. The dose and protocols were as previously described. Animals were evaluated on the 7th day of treatment using bovine serum albumin as standard. We used the open-field task to assess locomotor activity as described elsewhere. The covered distance was assessed for 30 min in an automatic arena. Locomotor activity was constantly monitored by an automated system installed in the arena.

**Forced swimming test**

The forced swimming test was conducted according to previous reports. During the test session, some behavioral parameters were recorded in seconds, such as the immobility, climbing and the swimming.

**BDNF measurement**

BDNF levels in the hippocampus, striatum, amygdala, and prefrontal cortex were determined by sandwich-ELISA using monoclonal antibodies specific for BDNF (R&D Systems, Minneapolis, United States). Total protein was measured by Bradford’s method using bovine serum albumin as standard.

**Statistical analysis**

Evaluation of study variables showed that normal-distribution parametric tests would be most appropriate. Differences among experimental groups in the forced swimming and open-field tests and in the assessment of BDNF levels were determined by one-way analysis of variance (ANOVA), followed by a Tukey post-hoc test when ANOVA findings were significant, and are presented as mean ± standard error of the mean (SEM). All analyses were performed using SPSS version 18.0.

**Results**

Administration of ketamine at a dose of 25 mg/kg over 7 consecutive days induced an increase of the covered distance in rats evaluated 1 hour after the last injection in all the periods studied, as compared with rats that received saline (Figure 1A; p < 0.05). On the other hand, the locomotor effect of ketamine was decreased in rats evaluated 6 hours after the last injection in 5 and 10 min, as compared with the saline group (Figure 1A; p < 0.05). The evaluation period was divided into blocks of 5 minutes, as shown in Figure 1A.

In the forced swimming test, 1 hour after the last injection of ketamine, there were no alterations in the immobility, swimming or climbing times (Figure 1B). However, 6 hours after the last injection, there was a decrease in immobility time (Figure 1B; p < 0.05) compared with the saline group. Furthermore, 6 hours after the last injection of ketamine, there was an increase in climbing time (Figure 1B; F(6,40) = 8.69; p < 0.001) as compared with the saline and ketamine groups evaluated 1 hour after the last injection. There was no alteration in swimming time (Figure 1B; F(6,40) = 0.46; p > 0.05).

Figure 2 illustrates the effects of ketamine administration on BDNF protein levels in the rat brain. When the animals were killed 6 hours after the last injection of ketamine, there was a significant decrease in BDNF protein levels in the prefrontal cortex (p < 0.001) and amygdala (p = 0.012), compared to the saline and ketamine groups that were killed 1 hour after the last injection. In the hippocampus, there was a decrease in BDNF protein levels when the rats were killed 1 or 6 hours after the last injection of ketamine (p = 0.007) as compared with the saline group. In the striatum, BDNF protein levels were reduced when the rats were killed 6 hours after the last injection (p = 0.048) as compared to the saline group.

**Discussion**

The effects of NMDA antagonists have been explored in rodents. In keeping with our results, Irifune et al. showed that ketamine induced a marked hyperlocomotion in rodents that dissipated 80 min after drug administration and was associated with an indirect dopaminergic agonist action in the nucleus accumbens. We also observed a decrease in locomotor activity 6 hours after the last injection of ketamine. The effects
observed may be explained, at least in part, by the fact that repeated ketamine administration produces a reduction in motivation, suggesting that ketamine induces both positive and negative effects on locomotor activity depending on the time elapsed between the last injection and evaluation.

In the present study, we found decreased immobility time and increased climbing time 6 hours after the last injection of ketamine (but not 1 hour after last injection). In contrast, other studies have shown that chronic treatment with phencyclidine (PCP), an NMDA receptor antagonist, decreased spontaneous locomotor activity and enhanced the forced swim-induced immobility. However, in keeping with our data, another study has shown that acute treatment with ketamine at the doses of 5, 10 and 20 mg/kg increased locomotor activity and decreased immobility time in the forced swimming and tail suspension tests in mice, suggesting that the effects exerted by ketamine are dependent on timing of administration and dose. In fact, ketamine has been shown to induce psychosis in an age-dependent manner.

Preclinical studies have shown that ketamine decreases the immobility time in the forced swimming test after acute or chronic treatment. Furthermore, in rodents subjected to the animal models of depression, ketamine has shown efficacy as an antidepressant. Clinical studies demonstrated that the administration of ketamine ameliorates depressive symptoms in patients with major depression. Interestingly, in the present study, we showed an antidepressant effect exerted by ketamine...
ketamine only 6 hours after the last injection, at which time motor activity was not increased. Previous studies from our laboratory evaluating the antidepressant effects of ketamine showed an antidepressant effect without affecting spontaneous locomotor activity. Moreover, acute, but not chronic treatment with ketamine at a dose of 15 mg/kg increased BDNF protein levels in the rat hippocampus, suggesting that these effects were not detectable at a later time because of adaptive mechanisms or due to the development of tolerance to the effects of ketamine on hippocampal BDNF levels.

Liu et al. recently demonstrated an increase in the expression of NMDA receptor NR1 subunits and pro-apoptotic genes, decreased anti-apoptotic genes, as well as altered expression of BDNF and exacerbated neurodegeneration in developing brains exposed repeatedly to PCP, subsequently resulting in the abnormal development of neurons and neuronal networks, which might be critical for schizophrenia symptoms in adulthood. Complex studies have shown that BDNF plays a role in schizophrenia. In fact, the neurodevelopmental abnormalities seen in schizophrenia could arise from abnormal cell migration, disconnection, disturbances in neurotransmitter systems, and changes in neural plasticity caused by alterations in the expression and/or functioning of neurotrophins. Carlino et al. found a slight reduction in BDNF levels using ELISA assay in the serum of patients with schizophrenia, and an increase in pro-BDNF, mat-BDNF and truncated-BDNF using Western blot analysis. In addition, subjects with schizophrenia had significantly lower BDNF levels in the prefrontal cortex and cerebrospinal fluid (CSF), suggesting that a deficiency in pro-BDNF processing may be a possible biological mechanism underlying schizophrenia. Other studies also found a significant decrease in BDNF levels in the serum of patients with schizophrenia. Conversely, another study showed an increase in BDNF levels. In the present study, we found a decrease in BDNF protein levels in the hippocampus and amygdala in rats killed 6 hours after the last injection of ketamine as compared with the saline group and with the group that was killed 1 hour after the last injection. BDNF levels in the striatum were also decreased in comparison with the saline group among rats killed 6 hours after the last injection, and levels in the hippocampus were decreased in the groups that were killed 1 and 6 hours after the last injection as compared with the saline group. Therefore, the effects of ketamine on BDNF levels were associated with the time at which the animals were evaluated after the last injection.

It is important to note that we chose the prefrontal cortex, hippocampus, striatum and amygdala as areas of interest in the current study because these brain areas are implicated in neuropsychiatric disorders. A clinical study showed that BDNF protein was altered in the hippocampus but unchanged in the prefrontal cortex of schizophrenia patients. Additionally, neonatal ventral hippocampal lesion, used as an animal model of schizophrenia, caused a decrease in BDNF expression in the hippocampus and a tendency toward reduction in the prefrontal cortex. Kang & Schuman showed that BDNF protein levels may cause changes in the strength of synaptic transmission in the hippocampus. On the other hand, unregulated BDNF levels in the hippocampus can induce other schizophrenia-like behavior. Furthermore, Hasbi et al. showed that the striatum, which is a dopaminergic area, regulated neuronal growth and maturation through BDNF. Thus, it is possible that locomotor hyperactivity be related, at least in part, with alterations in the hippocampus, striatum, prefrontal cortex, or amygdala in the current study. Studies from our group showed that acute administration of ketamine at a dose of 15 mg/kg reduced immobility time and increased BDNF protein levels in the hippocampus, but chronic administration did not exert this effect. The increase in BDNF protein levels may have been a defense mechanism. Indeed, animal models of schizophrenia induced by MK-801 administration increased BDNF levels, suggesting a protective response induced by cytotoxic insults.

In conclusion, chronic administration of ketamine altered behavioral parameters and BDNF protein levels in the rat brain, suggesting that both may be related, at least in part, to the effects of ketamine on the NMDA receptor. However, it is important to note that ketamine also acts on other systems, such as the monoaminergic and muscarinic receptors and voltage sensitive Ca channels, which are also involved in neuropsychiatric diseases; thus, the effects of ketamine could be related to synergistic effects on all of these pathways. Future studies evaluating the mechanisms by which ketamine exerts different molecular and behavioral effects in relation to the time elapsed after administration would be welcome to expand on the results reported herein.

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Disclosure
The authors report no conflicts of interest.

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