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ACTION OF KETAMINE ON GAMMA AMINOBUTYRIC ACID RECEPTORS IN THE EXPERIMENTAL MODEL OF DEPRESSION

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Abstract: According to the World Health Organization (WHO), more than 350 million individuals are affected by depression, being one of the main causes of disability worldwide. Recent studies suggest a deregulation of gamma-aminobutyric acid (GABA) levels, the main inhibitory neurotransmitter amino acid of the central nervous system (CNS), in the pathophysiology of depression. Furthermore, it has been postulated that nitric oxide (NO) released during glutamate uptake is related to depression. Thus, the inhibition of the nitrergic pathway would be related to the activation of GABAergic receptors (GABA A subtype), which could be one of the mechanisms responsible for the antidepressant action of compounds that modulate the activity of these receptors. In this context, studies have shown that ketamine (NMDA receptor antagonist) has antidepressant and anxiolytic effects in behavioral tests. Thus, the present study evaluated the participation of GABA A and GABA B receptors in the antidepressant effect of ketamine on NO levels in the cerebral cortex of mice in the tail suspension test (TSC). Therefore, the research design has a quantitative experimental character to investigate the involvement of the GABAergic system in rats treated with a dose of ketamine (0.5 mg/kg) whose results were compared to those of control animals. The presented results demonstrated that the administration of ketamine produced an antidepressant effect on TSC. These results indicate that the effect of ketamine on TSC may involve an activation of GABA A receptors and a possible inhibition of GABA B receptors. ketamine + L-arginine. Thus, we conclude that the antidepressant effect of ketamine involves a modulation of the GABAergic system and NO levels in the cerebral cortex.

Keywords: Depression, Ketamine, GABA, Nitric Oxide.

INTRODUCTION

Ketamine is a fast-acting antidepressant capable of promoting an increase in GABA levels in the anterior cingulate gyrus in rats subjected to unpredictable chronic stress and also in the medial prefrontal cortex of depressed patients. antidepressant effect of this compound, as well as to verify if this modulation is able to affect the activity of the nitrergic pathway. Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the CNS, so that most cell membranes of neurons and astrocytes express receptors for this neurotransmitter (SIVILOTTI, 1991).

GABAergic receptors participate in the modulation of several neural circuits and functions, including key stages of cell development and proliferation. The synthesis of GABA is carried out by the enzyme glutamic acid decarboxylase (GAD), which catalyzes the decarboxylation of glutamate to GABA in GABAergic interneurons (MARTIN, 1993).

GABA exerts its effects by modulating the activity of two types of receptors: ionotropic and metabotropic. Ionotropic receptors are made up of multiple subunits of membrane proteins that bind GABA and open a chloride-permeable ion channel. Metabotropic GABAergic receptors, on the other hand, are structures. Data from the literature suggest that a dysregulation of GABA levels may be involved in the pathophysiology of depression (PEHRSON, 2015).

In this context, it has been reported that depressed individuals may have a decrease in GABA levels in different brain regions. It is important to emphasize that GABAergic interneurons exert a significant influence on the neural networks that are responsible for the control of mood and cognitive function, so that the normalization of GABAergic neurotransmission promotes an improvement in the cognitive deficits observed in individuals

with depression (PEHRSON, 2015).

On the other hand, it is important to emphasize that the participation of GABAergic receptors in depression is not fully understood, but there is evidence that the modulation of the activity of GABA A receptors is related to an antidepressant effect, since the lack of expression of GABA A receptors are related to depressive phenotype (VOLLENWEIDER et al., 2011).

GABAergic receptors of the GABA B subtype also seem to be related to the pathophysiology of depression, since preclinical studies show that the suppression of the expression of functional GABA B receptors causes rodents to present an antidepressant phenotype. GABA B is capable of promoting an antidepressant response in pre-clinical trials (MOMBEREAU et al., 2005; NOWAK et al., 2006; FELICE et al., 2012).

It is important to emphasize that GABAergic interneurons exert a significant influence on the neural networks responsible for the control of mood and cognitive function, so that the normalization of GABAergic neurotransmission promotes an improvement in the cognitive deficits observed in individuals with depression (PEHRSON, 2015).

In addition, evidence shows that the GABAergic system is implicated in the rapid antidepressant effect of ketamine (an NMDA receptor antagonist), which is very effective for the treatment of depression in patients refractory to pharmacotherapy with classic antidepressants (BERMAN et al., 2000; ZARATE et al., 2006).

Given the above, the present study evaluated the participation of GABA A and GABA B receptors in the antidepressant effect of ketamine on NO levels in the cerebral cortex of mice in the tail suspension test (TSC).

MATERIALS AND METHODS

In this study, 60 rats were used, divided into three groups of 20 animals kept in normal vivarium conditions, temperature of 25° with water and food ad libitium and light/dark cycle 12/12 hours.

EXPERIMENTAL GROUPS

1. Group control
2. Ketamine Group
3. Group: Ketamine + L-arginine

The animals were acclimatized in the place where the experiment was carried out 24 hours before the behavioral tests. All procedures performed were approved by the Ethics in Animal Use Committee – ULBRA.

TAIL SUSPENSION TEST (TSC)

This test is based on the principle that animals subjected to inescapable stress develop a posture of immobility, which is reduced by interventions such as treatment with antidepressants.

In this test, mice were suspended by their tails 50 cm above the floor using adhesive tape and immobility was recorded for 8 minutes.

OPEN FIELD TEST (ACT)

The mice were submitted to one session in the open field test, 60 minutes after the administration of the compounds. The test was carried out in a wooden box measuring 40 x 60 x 50 cm high, with the floor divided into 12 equal squares. The number of crossings performed with all four paws was recorded in a 6-minute session.

TAIL SUSPENSION TEST

The TSC is a predictive test for antidepressant activity, in which mice when suspended by the tail adopt a posture of immobility in the face of this inescapable situation. The immobility time is measured over a period of 6 minutes, so a reduction in it is considered an antidepressant-like effect.

DETERMINATION OF OXIDATIVE STRESS

THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS)

The TBARS technique consists of heating the homogenate with thiobarbituric acid and the consequent formation of a colored product. The appearance of color occurs due to the presence of malondialdehyde from lipid peroxidation in the biological material.

Tissue samples were placed in test tubes with a mixture of trichloroacetic acid (TCA), 10%, and thiobarbituric acid (TBA), 0.67%. Subsequently, they were heated in a bath for 30 minutes and cooled on ice for five minutes. TBA reacted with lipoperoxidation products forming a Schiff base, TCA was used to denature the proteins present, in addition to acidifying the reaction medium. After cooling the samples, 1.5 ml of n-butyl alcohol was added to extract the formed pigment, placed in a shaker for 45 seconds and centrifuged for 10 minutes at 3000 rpm. Finally, the colored product, present in the upper fraction, was read in a spectrophotometer with a wavelength of 535 nm. The concentration of TBARS obtained was expressed in nmol per milligram of protein.

EVALUATION OF NITRIC OXIDE (NO) METABOLITES

After carrying out the behavioral tests (TSC and TCA), the animals were decapitated to remove the cerebral cortex, the structure used to measure NO levels.

Briefly, cerebral cortex homogenates from mice treated with ketamine and muscimol were mixed with 25% trichloroacetic acid and centrifuged for 10 min, and the supernatants were neutralized with potassium bicarbonate. After enzymatic conversion by nitrate reductase, nitrite was measured using the Griess colorimetric reaction in a microplate reader at a wavelength of 540 nm. The Griess reaction is based on the enzymatic reduction of nitrite to nitrates in the presence of nitrate reductase and NADPH.

RESULTS

The results show the effect of ketamine administration on NO content in the cerebral cortex of mice. The post hoc test revealed an increase in NO in animals treated at a dose of 0.5 mg/kg of ketamine.

Duncan's post hoc test revealed a synergistic antidepressant effect induced by ketamine administration. There was also a significant effect on the time of immobility in the TSC in the group of animals that received the L-arginine + ketamine combination, in relation to the control group.

Duncan's post hoc test showed an increase in NO content in the ketamine-treated group compared to the control group. On the other hand, a reduction of NO was observed in the groups treated with muscimol alone and with L-arginine + ketamine. On the other hand, administration in the group treated with 0.5mg/kg of ketamine decreased lipoperoxidation (LPO) as well as in the group treated with L-arginine (800mg/Kg) + ketamine (0.5mg/Kg).

A synergistic antidepressant effect was observed when a dose of ketamine was administered to rats subjected to TSC, suggesting that the antidepressant effect may be related to the activation of GABA A receptors.

DISCUSSION

Clinical studies have found that a single infusion of ketamine (0.5 mg/kg) administered intravenously over a period of 40 minutes reduces the clinical manifestations of depression for approximately one week (ZARATE JR. et al., 2006). The antidepressant effects of ketamine appear about four hours after its intravenous administration

(ABDALLAH et al., 2016).

A recent study demonstrated that a low dose of ketamine combined with propofol improves antidepressant efficacy due to increased phosphorylation of GABA A receptors in the hippocampus of stressed rats (CHEN et al., 2015).

Evidence indicates that the GABAergic system is implicated in the rapid antidepressant effect of ketamine (NMDA receptor antagonist) in patients refractory to classic antidepressants (BERMAN et al., 2000; ZARATE et al., 2006).

In another study, with the same dose and route of administration, ketamine was effective in increasing the NO content in the cerebral cortex and hippocampus of mice (CUNHA et al., 2015).

Singh et al. (2016), after a double-blind study containing 67 participants received ketamine twice a week. Finally, the study also revealed that the administration of two or three doses of ketamine, at 0.5 mg/kg in one week, maintained an antidepressant effect for 15 days.

The enantiomer known as esketamine showed three times greater antidepressant activity when compared to R-ketamine; this occurred because esketamine has about four times greater affinity for glutamatergic NMDA receptors when compared to R-ketamine (DOMINO, 2010).

A randomized double-blind study performed by Singh et al. (2016) demonstrated that the intravenous administration of esketamine at doses of 20 and 40 mg/kg showed a significant improvement in the clinical manifestation of this disease when compared to the placebo group. Recently, in a series of case reports, evidence of the safety and efficacy of esketamine for the treatment of major depressive disorder with psychotic features, when used subcutaneously, is presented (AJUB, 2018).

According to Cao et al. (2019), based on a trial involving 55 patients, divided into a group that received a placebo (control group) and two other groups that received ketamine infusions (Group A: 0.5 mg/kg of ketamine; Group B: 0.2 mg/kg), a considerable decrease in depression was reported in group A, probably due to the dose of 0.5 mg/kg. For the control group, no significant changes were noted.

In the study by Mathew et al.15 (2019), with 400 patients divided into groups treated with ketamine, 120 participants from each group were classified as responders to the respective treatments. On the other hand, at high doses, it may be associated with cognitive impairment.

The correlation analysis did not show a direct relationship between the increase in NO and the time of immobility in the TSC, reinforcing the hypothesis that NO has a dual role in mood modulation. Thus, it has already been demonstrated that the effects on mood modulation may involve both a decrease and an increase of NO in brain regions.

Corroborating this hypothesis, Cunha and Collaborators (2015) analyzed that the antidepressant effect involves an inhibition of the activation of NMDA receptors and a subsequent decrease in nitrite, as well as the treatment of ketamine, which have an anti-immobility effect on the TSC, promoting an increase in NO both in the hippocampus as in the cerebral cortex in rats.

Recently published data suggest that the inhibition of the nitrergic pathway would be related to the activation of GABAergic receptors of the GABA A subtype, which could be the mechanism responsible for the antidepressant activity. effect promoted by the administration of subactive doses of muscimol with ketamine (SARAVI et al., 2016).

A synergistic antidepressant effect resulting from the administration of L-arginine and

ketamine was also observed, promoting a decrease in the time of immobility in the CST. These data are similar to studies that showed that administration of low doses of NO precursors and subactive doses of NO inhibitors are capable of promoting antidepressant activity (HEINZEN, 2002).

Initially, a synergistic antidepressant effect was observed when a dose of ketamine was administered in rats submitted to TSC. These data suggest that the antidepressant effect of these compounds may be related to the activation of GABA A receptors.

Thus, we can hypothesize that ketamine regulates brain NO levels by inhibiting NMDA receptors promoting the increase of NO from nitrates and nitrites.

However, this hypothesis needs further experiments to be confirmed.

FINAL CONSIDERATIONS

Data from the literature dealing with the GABAergic system in depression point to an activation of GABA A receptors and blockade of GABA B receptors as a mechanism involved in the antidepressant effects.

The present study reinforces the GABAergic system as a target for antidepressant agents under development and extends data from the literature regarding the mechanisms underlying the antidepressant action of ketamine, suggesting that its effects on the TSC may be dependent on the activation of GABA A receptors and a possible inhibition of GABA B receptors.

Finally, it is emphasized that, despite its promising effects, further research on the subject is valid, in order to establish other dosage characteristics of ketamine against depression, such as the number of weekly infusions and the period of use necessary for a therapeutic result effective.

Based on what was described above, it is hypothesized that ketamine may contribute to inhibiting the molecular cascades involved in depression.

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