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ANTIDEPRESSANT EFFECT OF KETAMINE IN BEHAVIORAL MODELS WITH AGED RATS

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ABSTRACT

The aim was to evaluate the action in behavioral models of ketamine as antidepressant drugs in aged rats. Thirty rats were divided into three groups. One group received treatment with imipramine hydrochloride 20mg/kg (i.m.). In the second, we administered ketamine (20mg/kg/i.m.), single dose, and the third group / control, received saline 20 mg / kg (i.m.). The antidepressant activity was evaluated using the forced swim test and to exclude false-positive results, was employed one additional test (OF). The results showed that in the forced swim test, the Ketamine and imipramine significantly decreased the immobility time when compared to saline group, indicating an antidepressant effect of ketamine compared to saline group. The parameters of movement and stillness are proven by the group of animals that received imipramine. There was no stimulatory effect of drugs administered in the open field test.

Keywords: **Depression, Ketamine, Rats *Wistar***

INTRODUCTION

Depression is the fourth cause of disease in the general population. It is estimated that in 2020, it will be indexed, among all other diseases, as the second highest incidence in the world. Several studies have shown that up to 23% of elderly suffering from depression or depressive symptoms, which, in turn, affect the quality of life of the elderly **[1]**.

Depression is common in old aged people and, in some cases, improves with adequate antidepressant treatment, decreasing the morbidity and mortality caused by the disease **[2, 3]**. Among the therapeutic strategies, there are the inhibitors of the reuptake of biogenic amines such as tricyclic antidepressants and the selective serotonin 5-HT reuptake inhibitors and NE, the monoamine oxidase inhibitors, dual inhibitors of 5-HT and NE and atypicals, that act as antagonists of 5-HT and its reuptake inhibitors **[4]**.

However, there are treatments for mood disorder that often take weeks or months to show their antidepressant effects, and a significant number of patients do not respond to treatment, even after months. Furthermore, the increase in suicide attempts have already constitutes a serious public health problem, especially during the first month of therapy with antidepressants standard.

Ketamine hydrochloride (an antagonist of N-methyl-D-aspartate) is commonly used as an anesthetic, but recently has been studied due its antidepressant action, resulting in a sudden and effective disappearance of depression symptoms **[5, 6, 7]**.

The NMDA is an ionotropicn receptor activated by glutamate, the most abundant excitatory neurotransmitter, permeable to calcium, sodium and potassium **[8]**. Thus, the study aimed to evaluate the action of the anesthetic ketamine, as antidepressant drugs in elderly mice in behavioral models, knowing that NMDA receptor antagonists, mimicking clinical effects of antidepressants.

MATERIAL AND METHODS

Animals

The study protocol and experimental design were approved by the Animal Ethics Committee of Univali. In total, 60 Wistar female rats 16 months of age (400-450g) were used, maintained with free access to food and water in the amount of 10 to 20 g / animal / day in light / dark cycle of 12/12 hours at 22°C ± 2. The animals were divided into three groups. One group received treatment with imipramine hydrochloride 20mg/kg (im) applied for 5 days from the pre-test. The second was administered ketamine (20mg/kg/im), single dose, applied after the pre-test and saline in the rest of the experimental period, the animals of the third group /control, received saline 20mg/kg (im) in the same period and conditions of de other groups.

The Forced Swimming Test (FST)

In accordance with Cryan *et al* **[9]** forced swimming is indicated when it is desired to test the effect of antidepressants in mice, being easily reproducible. This test was proposed by Porsolt et al **[10]** and subsequently altered by taking into account the parameters of immobility, swimming and climbing animals **[9, 11, 12]**, using an acrylic cylinder of 60 cm diameter and 25 to 30cm column of water, heated to 25°C. In the first stage, corresponding to the pre-test, the animals of all groups were subjected to sessions of swimming for 15 min/animal. Removed, they are dried with cloth towels and maintained under a 40 watt incandescent lamp at a distance sufficient to complete the drying comfort.

Twenty-four hours after the pretest, began the first test session during 5 minutes/animal, recording the behavior of rats in a digital camera Samsumg Imagine L70, for further annotation movements in a spreadsheet numbered 0 to 300 seconds. The second test was conducted after 120 hours of pre-test. The experiment was conducted in the period between 18:00 and 21:00 hours, considering the pace of biological species **[13]**. According to the literature, swimming takes is the horizontal movement of the animal, crossing from one quadrant to another or performing circular movements. When the animal explores the cylinder walls with forepaws in vertical movements, the parameter is climbing and immobility when no movements are observed, except those necessary for the animal to keep your head out of the water **[14]**.

Open Field Test (OF)

It is performed in a circular arena of PVC in 60cm X 54cm size, with a base demarcated in twelve quadrants. Two hours before the first and second forced swim test, animals are placed in the center of the open field while an observer recorded the frequency of movement (locomotion), ie, the number of times that the animal enters in one of the quadrants with all four paws and the number of rearings, which consists of standing on their hind legs or leaning against the wall. The test duration is 5 minutes/animal, cleaning the floor and walls of the arena with alcohol (10%) between each animal. The open field test is used to verify if the behaviors are resulted by stimulatory effects of the drug, comparing mainly the locomotor frequency between experimental and control groups.

Statistical Analysis

The tests dates of the modified forced swimming and open field, applied in all groups were treated by the Student t test, considered the significance of $p < 0.05$.

RESULTS

FST

The **Figure 1** shows that the administration of ketamine (20mg/kg)/single dose, decreased significantly ($p = 0.0001$) the duration of immobility as compared to the control group within 24 hours. Group imipramine, gave a ($p = 0.0067$), also in relation to control.

After 120 hours of pre-test, immobility as the parameter, the difference between groups (control and ketamine) was significant ($p = 0.0002$). Decreased immobility of imipramine group compared to the control, resulted in ($p = 0.0152$) **Figure 2**.

The **Tables 1 and 2** show that administration of ketamine and imipramine, did not result in behavioral and / or motor dysfunction in the open field test.

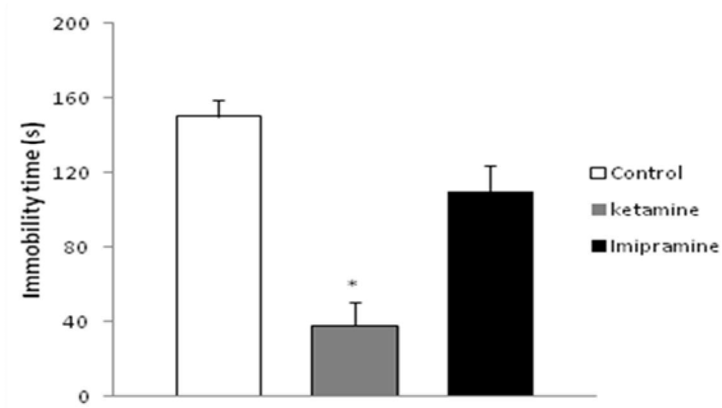

Figure 1: Effect of Ketamine on the Immobility Time at the First Forced Swimming Test (24 Hours After the Pre-Test). Values Represent the Mean_S.E. (*n*_10). **p*<0.05 *vs.* Control Group

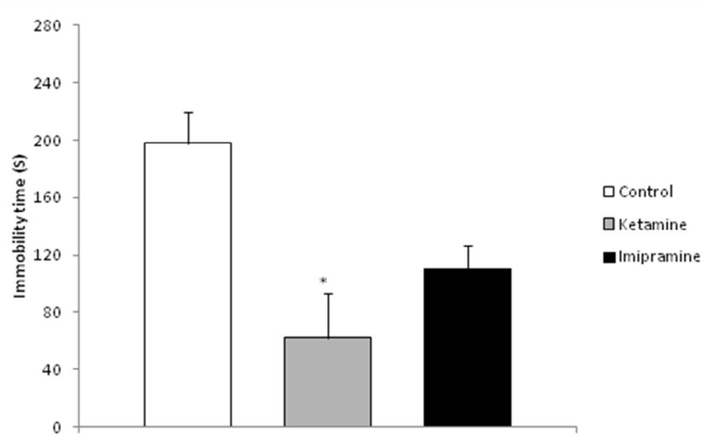

Figure 2: Effect of Ketamine on Immobility Time in the Second Forced Swim Test (120 h After the pre-test).Values Represent the Mean_S.E. (*n*_10). **p*<0.05 *vs.* Control Group

Table 1 - Locomotor Activity in the Open Field Test (24h after the pre-test)

Treatment	Locomotion	Rearing
Control	76 ± 17.99	12.8 ± 4.02
Ketamine 20mg/kg	48.1 ± 27.34	3.8 ± 4.47
Imipramine 20mg/kg	51.7 ± 22.15	5.2 ± 3.29

NOTE: The Behavioral Parameters were Recorded for 5 min. Locomotion: Number of line crossings. Rearing: number of Times Seen Standing on Hind Legs. Values Represent the Mean_S.D

Table 2: Locomotor Activity in the Open Field Test (120h after the pre-test)

Treatment	Locomotion	Rearing
Control	44.9 ± 21.3	3.6 ± 4.58
Ketamine 20mg/kg	26.5 ± 17.91	2.4 ± 2.01
Imipramine 20mg/kg	22.8 ± 4.32	0.3 ± 0.67

NOTE: The Behavioral Parameters Were Recorded for 5 min. Locomotion: Number of Line Crossings. Rearing: Number of Times Seen Standing on Hind Legs. Values Represent the Mean S.D

DISCUSSION

The aim of this study was to evaluate the antidepressant effect of ketamine (20 mg / kg) using animal models of behavior in aged rats. Animal models of depression are methods for detection and study of antidepressants neurobiology of depression. Although these models cannot reproduce symptoms of depression (because they are essentially human behavior), Wilnner **[13]** found that the model of chronic stress can induce in the animal a similar situation of dysthymia humans, besides causing anhedonia.

McKinney and Bunney **[15]** proposed that the animal models of depression must meet minimum requirements, such as: to present analogy with human depression in their manifestation or symptoms and promote behavioral changes in animals that can be controlled, ie, these changes are reversible with antidepressants. There are parameters for an animal model that has utility in preclinical evaluation, these should have predictive validity and reproducibility laboratory.

The forced swim test developed by Porsolt *et al.*, **[10]**, has been widely used to evaluated preclinical antidepressant activity, it is easy to handle, reproducibility in laboratories and possibility to detect broad spectrum of antidepressant drugs.

Many studies using that proven clinical efficacy of antidepressants, have the purpose of validating the model modified forced swim **[9, 11, 16, 17]**.

Ketamine is increasingly subject of research and study, after the administration in rats of a sub-dose of this substance, it induced changes in behavior patterns and parameters of bioactive amines (neurotransmitters) in these animals **[18]**.

In the present study, administration of ketamine significantly decreased the immobility time in the forced swimming test in both the first and the fifth day after the pre-tests, showing that a single dose of ketamine can promote rapid and lasting antidepressant effects. The decrease of immobility was also observed in imipramine group, significant relative to the control group in both tests. The use of a group by applying a known antidepressant (imipramine), has validated the parameters of movement (swimming, climbing) in relation to immobility, corroborating the literature. Furthermore, to exclude false-positive results it was used an additional test (OF), to evaluate the presence or absence of a motor hyperstimulation caused by the drug. Thus, the administration of ketamine and imipramine showed no abnormal behavior and / or any motor dysfunction.

Garcia *et al* **[19]** showed that acute administration of ketamine in doses 10 and 15 mg / kg as well as imprimamina (20 and 30 mg / kg) reduced immobility time compared to saline group, without affecting locomotor activity.

Zarate *et al.*, **[20]** described a human improvement in depressive symptoms with a single intravenous dose of ketamine (0.5 mg / kg) that induced a significant, rapid and prolonged effect. The basic symptoms of major depressive disorder were significantly attenuated after 24 hours of administration of ketamine, and the response rates obtained are comparable to those seen after six to eight weeks of treatment with monoaminergic antidepressants, highlighting the relevance of the results with the use of ketamine.

Chaturvedi *et al*., **[21]**, conducted a study using the induced shock in mice as a model of depression, evaluating them later by open field test and forced swimming test. The presentation of inescapable shock significantly reduced ambulation in the open field test and increased immobility time in the forced swimming test. However, after administration of ketamine, the symptoms of depression, induced by shock, decreased considerably in the forced swimming test. Thus, the authors conclude that drugs NMDA receptor antagonists possess antidepressant effect and also show a synergism with imipramine.

Krystal **[22]**, explains that one of the reasons that ketamine can act more quickly than the typical antidepressant drugs is due its effects that arise as a progressive adjustments to other antidepressants, including reducing NMDA receptor function of glutamate, induction of CREB transcription factor and brain-derived neurotrophic factor (BDNF) as well as improving the function of glutamate AMPA receptors. It is possible that drugs that directly target the CREB, BDNF and AMPA receptors may have antidepressant effects of rapid onset.

However, Yilmaz *et al*., **[23]** suggests that perhaps the antidepressant action of ketamine, demonstrated in this study may be related to decreased glutamatergic activation, particularly in the hippocampus and prefrontal cortex. NMDA receptor antagonists prevent long-term potentiation, impairing synaptic plasticity, and when they have an effect in the hippocampus undertake learning in rats **[24]**. Moreover, the study of Petty *et al*. **[25]** reinforces this question, as reported that, when performed intracortical microinjections of glutamate in the prefrontal cortex of Sprague-Dawley rats there were exacerbation of behavioral despair the model of learned helplessness, thus, blocking of NMDA glutamatergic receptors can reduce it.

CONCLUSION

According to the methodology used in this study, the results indicate antidepressant effect of ketamine compared to saline group. The parameters of movement and immobility are demonstrated by the group of animals receiving imipramine. There was no stimulatory effect of drugs administered in an open field.

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