

Research Article

Ameliorative effects of ketamine administration in experimental model of Chronic Unpredictable Mild Stress



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Abstract

Background: The etiology of depression is still unclear, but multiple studies explained several theories attempting to describe the development of depression. Depressive symptoms are usually combined with morphological and neuroendocrine deficits in the mesolimbic system including hippocampus. One drug or combination of more than one are associated with high rate of non-response, partial, or delayed response onset from weeks to months. Thus, the need for new and better treatment is mandatory. Purpose of the study: to assess the mechanisms and effects of a single dose of *ketamine* injection in experimental model of chronic unpredictable mild stress (CUMS). **Methods:** thirty rats were separated into 3 groups: control (C), CUMS group in which rats faced different physical stressors for six weeks, Ketamine treated CUMS group in which rats faced different physical stressors for six weeks then every rat received a single sub-anesthetic intraperitoneal injection (i.p) of ketamine at a dose of 10 mg/kg, 24 hours prior to the end of the experiment. After the end of the CUMS period, before and after ketamine administration, behavioral test was executed as sucrose preference test. In addition, serotonin concentration was measured in hippocampus of the dissected rat brain. **Results:** the administration of single i.p injection of ketamine significantly increased sucrose preference test and hippocampal serotonin level as compared to CUMS group but still significantly lower than the control group. **Conclusion:** Chronic stress exposure induced many brain damaging events and the administration of ketamine ameliorating the depressive symptoms.

Keywords: CUMS, ketamine, sucrose preference test.

Introduction

Depression, also known as major depressive disorders, is one of the commonest psychiatric disorders and causes of disability across the world. It presents a serious socio-medical burden that will become more serious in the coming years according to WHO expectation^[1]. Symptoms of depression vary according to its severity, spread to many domains of life affecting person's emotional, social, occupational functioning. These symptoms show changes in mood, anxiety, memory loss, anhedonia, optimism, sleep change rhythm,

energy loss, appetite change, loss of libido, and psychomotor activity^[2].

The treatment plans for depression consist of pharmacological and non-pharmacological strategies containing psychotherapeutic approach as cognitive behavioral, relaxation and imagery techniques, acupuncture with massage and nutritional supplements. Forty percent of patients do not respond adequately to currently available anti-depressant medications^[3], so they open the way for new medications to develop and assist in reducing the progression of depression and its possible complications^[4].

The aim of the current study is to induce depression in adult male albino rats and to study in them some pathophysiological changes. Cumulatively, the present study showed the effect of treatment by a single sub-anesthetic ketamine dose on pathophysiological changes associated with depression.

Materials and methods

I- Animals;

Thirty adult male albino rats of national strains with average weight 200 ± 20 gm were gained from the National Research Center, Cairo, Egypt. They were housed in well ventilated cages made of stainless steel cages at normal room temperature with normal dark and light sequences. They were left for two weeks after arrival for acclimatization. Rats were fed a commercial rat's diet (Nile Company, Egypt) and tap water ad libitum through the time of the study except the timing of food and water deprivation in stressor's groups. All experiments were performed according under the appropriate animal licenses No.323:4/2022 permitted by the animal care committee of Faculty of Medicine-Minia University, according to the international guidelines.

II- Experimental design;

Rats were categorized into the following three equal groups of ten rats each:

- 1) **control group (C):** in which rats were allowed freely wandering in their cages with free access to food and water.
- 2) **Chronic unpredictable mild stress group (CUMS):** in which rats faced different physical stressors for six weeks. Stressors like water deprivation, food deprivation, noise. These stressors were administered daily and to have the unpredictable procedure, two stressors were applied daily in random manner^[5].
- 3) **Ketamine treated CUMS group (K. CUMS):** in which rats were exposed to different physical stressors for six weeks^[5] then every rat received a single sub-anesthetic intraperitoneal injection of ketamine at a dose of 10 mg/kg, 24 hours prior to the end of the experiment^[6].

III- Behavioral test;

After the end of the CUMS period, before and after ketamine administration, sucrose preference test was executed as follow: rats were

singly housed in a cage after a 12 hours' duration of food and water withdrawal. After that, all rats were given 2 bottles containing water or 2% sucrose solution. Sucrose and water bottles were put and assigned randomly in sides of every cage, Sucrose preference test includes two sections: an initial for 24 hours before drug administration (the baseline sucrose test) then for 2 hours after drug administration (the sucrose preference test). The weights of water and sucrose consumed were measured according the following equation: Sucrose solution (ml)/ (Sucrose solution [ml] + water [ml]) $\times 100\%$ ^[7].

IV- Sample collection;

At the end of the whole experimental design, and after the end of second section of behavioral test, the surviving rats were sacrificed by cervical dislocation. The heads of sacrificed rats were rapidly dissected carefully and the brains were removed in gentle way. Brain was used to expose the hippocampus which was identified as a C shaped structure in coronal section. Then the hippocampal brain tissue was dissected, weighed, homogenized to assay serotonin that was determined spectrofluorometrically as Ciarlone (1978)^[8] by using spectrofluorometer (Shimaduz RF- 5000), Japan).

Statistical analysis of data:

The data were expressed as the means \pm Standard deviation(SD), comparisons between different groups were performed by one-way analysis of variance (one-way ANOVA), then the least significant difference (LSD) test. Results with $p < 0.001$ were considered statistically significant. Statistics were analyzed using IBM SPSS 28.0 statistical package software (IBM; Armonk, New York, USA).

Results

I- Effect of CUMS with or without ketamine on sucrose preference tests:

Table (1) show that CUMS reduced the sucrose tests as compared with control group in both the baseline (24 h) and the preference tests (2 h). No significant difference in baseline sucrose test before drug administration was seen between all stressed groups as compared with the stressed group (CUMS). Concerning sucrose preference test, the ketamine treated CUMS group showed a significant higher

sucrose preference test as compared to CUMS group but still significantly lower than the control group.

group showed significant lowest levels among all experimental groups. On the other hand, the hippocampal serotonin levels were significant higher in ketamine treated CUMS as compared to CUMS group but still lower than control group as shown in table (2).

II- Effect of CUMS with or without ketamine on hippocampal Serotonin level:

The results of the current study exhibited that the hippocampal serotonin level in CUMS

Table (1): Effect of CUMS with or without treatment on sucrose preference tests:

		c	CUMS	K. CUMS	P value
		N=10	N=10	N=10	
Sucrose preference test 24 hrs	Mean ± SD	74.6±2.3	47.2±1.8 ^a	45.1±2.3 ^a	<0.001*
Sucrose preference test 2 hrs	Mean ± SD	53.2±9.4	25.8±2 ^a	33.7±1.4 ^b	<0.001*
P value 24 hrs vs 2 hrs		<0.001*	<0.001*	<0.001*	

Data are expressed as Mean±SD. The mean difference between groups was considered statistically significant when P value < 0.001. c: Control group, Chronic unpredictable mild stress group (CUMS), K. CUMS: Ketamine treated CUMS group.

- ^a significant with the corresponding mean of the control group.
- ^b significant with the corresponding mean of the CUMS group.

Table (2): Effect of CUMS with or without treatment on hippocampal Serotonin level:

		c	CUMS	K.CUMS	P value
		N=10	N=10	N=10	
Serotonin	Mean ± SD	16.8±0.7	3.6±0.7 ^a	6.4±0.7 ^b	<0.001*

Data are expressed as Mean±SD. The mean difference between groups was considered statistically significant when P value < 0.001. c: control group, Chronic unpredictable mild stress group (CUMS), K. CUMS: Ketamine treated CUMS group.

- ^a significant with the corresponding mean of the control group.
- ^b significant with the corresponding mean of the CUMS group.

Discussion

Depression is one of the most prevalent psychiatric illness in the world. Nearly 300 million people around the world suffer from depressive episode in their lives which equal to 4.4% of the world’s population^[9].

tests simulating different unpredictable life stressors which elicit emotional and behavioral responses contributing to neuropsychiatric disorders, such as major depressive disorders in humans^{[11], [12]}.

Depression in animals can be induced by numbers of behaviorally tests, based mainly on social and environmental reinforcement with a good credibility and validity^[10]. Despite the wide variety of depressive-inducing protocols, the chronic unpredictable mild stress (CUMS) animal model was chosen in the present study as it is one of the most accepted behavioral models. In CUMS, the laboratory animals faced external, variable, different and unpredictable

The mesolimbic system is also known as the reward system that is responsible for emotional expression, learning, behavioral response and other motivational cognition. Chronic stress induces reactive oxygen species (ROS) that cross BBB to change mesolimbic system activity. Depression is very common with its anhedonia behavior. CUMS model is obviously known as anhedonia-inducing potential^[13]. In the present study, the investigation of the depressive and anhedonia like behaviors was

done by sucrose preference test (SPT). The CUMS group significantly decreased SPT as chronic stress causes dendritic remodeling in prefrontal cortex as well as functional changes in behavioral response^[14].

The present results demonstrated that the sucrose preference test was significant higher in ketamine treated CUMS groups as compared with CUMS group. Kingir and his colleagues 2023 explained the anti-depressant action of ketamine as they modulate the neurotransmission in mesolimbic system affecting behavioral response in animal^[15].

Regarding to the monoamine theory, Monoamine oxidase (MAO) density is elevated during episodes of depression. This results in greater metabolism of catecholamines, such as serotonin (5-HT) as shown in the present study. Catecholamines usually participate in mental activities and emotional control in body and it is confirmed to related directly to the onset, pathophysiology and symptoms of depression^[16]. Additionally, the serotonin deficiency hypothesis confirmed multiple abnormalities of serotonin receptors with a decrease of its availability in multiple brain areas of patients with MDD, which regulates serotonin function in brain^[17].

On the other hand, ketamine administration significantly increases hippocampal serotonin level by acting through AMPA receptors, which is localized to serotonergic neurons eventually arouses serotonin secretion by stimulating 5-HT_{1A} receptors and 5 hydroxy tryptaminergic transmission in the hippocampal^[18].

Conclusion

The current study established that the experimental model of CUMS in rats is characterized by anhedonia behavior with decreasing the hippocampal serotonin level. Ketamine was in clinical use since 1970 as analgesic and with anesthetic characters. In addition, the present study showed that the single sub-anesthetic intraperitoneal injection of ketamine at a dose of 10 mg/kg produced a fast-acting antidepressant response in CUMS model.

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