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Effects of Chronic Ketamine Exposure on Habenula Nucleus: A Histomorphometric and Stereological Study

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Research Article

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ABSTRACT

Introduction: Ketamine is an anesthetic drug that has been recently used in the treatment of mood disorders. The aim of this study was to examine the effects of chronic use of ketamine on the neuroglial elements of Habenula nucleus.

Materials and methods: Male wistar rats (8 weeks old) (200 ± 20 gr) were divided into 2 groups (N=10) namely experimental and control. Experimental group received ketamine at dose of 10 mg/kg intraperitoneally for one week. The control animals were treated with saline. The brains were processed and stained with H and E. The bilateral surface of the HB were defined and measured with software. The number of astrocytes and dark neurons were counted on both sides according to the modified stereology method.

Results: The number of dark neurons in the medial Habenula HB of experimental group (15 ± 4) that showed significant difference in comparison with those of control. The wet weight of the adrenal gland in the experimental group (42.5 ± 7 mg) showed significant level of difference in comparison with those of control (18 ± 1.20 mg) ($P<0.05$). The weight of wet brain (the brain stem and cerebrum were removed) in ketamine group (1.28 ± 0.04 gr) showed meaningful difference in comparison with control (1.71 ± 2 gr) ($P<0.05$). Histomorphometry of the HB in the experimental animals showed no meaningful difference with those of the control animals ($p>0.05$). The number of counted astrocytes in experimental group showed significant difference in comparison to those of the control group ($P<0.05$).

Conclusion: The results of this study revealed that chronic exposure to ketamine is not associated with histomorphometric changes in the HB. Furthermore, our results showed that chronic use of ketamine leads to reactive gliosis and neuronal death in the medial HB. The ketamine induced neurodegeneration was associated with decreased brain weight and adrenal hypertrophy.

INTRODUCTION

Ketamine, a phencyclidine derivative, is an anesthetic drug that is used in dissociative anesthesia. Ketamine has been shown to have an antidepressant effect in animal models [1]. In this context ketamine has received the attention because of its antidepressant effects in human [2]. Due to its psychedelic effects, ketamine is abused as a recreational substance [3]. Studies have shown that prolonged use of ketamine can cause various central nervous system complications [4-6]. Furthermore, increase in the levels of proinflammatory cytokines like IL–6 and IL–1 β, protein damage and lipid peroxidation in the hippocampus suggesting ketamine may lead to cognitive impairment, behavioral abnormalities and neurodegeneration [7]. One of the critical region in emotional processing is Habenula. The Habenula (HB) is a part of Epithalamus and it is known that this bilateral nucleus plays a pivotal role in sleep/wakefulness and processing of stress and fear provoking stimuli [8,9]. The HB is composed of medial and lateral parts, each having distinct functions, structure and anatomical connection [10]. Studies have shown that HB lesion leads to schizophrenic-like symptoms such as cognitive impairment [11]. Additionally, there are reports on the Habenular dysfunction in the development of the mental health disorders [12]. Although studies have reported that ketamine could relieve rapidly depression by blocking bursting in HB [13]. Previous reports have documented evidence that acute ketamine use induces tau hyper phosphorylation which is known as a hallmark of Alzheimer [14]. It has been proposed that ketamine adverse effect on the central nervous system is mainly mediated through the oxidative stress process [15]. Increased level of oxidative stress leads to the activation of Hypothalamo-Pituitary-Adrenal (HPA) axis, neuronal death pathways and gliosis. Stressful conditions lead to increased level of glucocorticoids, triggering neuronal death and subsequently gliosis. With regard to the role of astrocytes in neuronal homeostasis and antioxidant production in the CNS in one hand and ketamine effects on stress level, we hypothesized that ketamine chronic exposure may lead to neuroglial alterations in HB. Given the little data on effects of chronic ketamine exposure on the HB we aimed to examine the effects of chronic use of ketamine on the neuroglial elements of HB with histomorphometry and stereology methods.

MATERIALS AND METHODS

.All the experiments in this study were conducted in educational and research laboratory of neuroscience of North Khorasan University of Medical Sciences (NKUMS). Male wistar rats (8 weeks old) (200 ± 20 gr) were divided into 2 groups (N=10) namely experimental and control. Experiments were done during the light period of cycle in accordance with NKUMS animal ethic committee. Experimental group received ketamine at dose of 10 mg/kg intraperitoneally for one week. The control animals were only treated with saline. At the end of experiment animals were deeply anesthetized with chloroform and animals were perfused transcardially according to our previous studies. The animals were kept at 4˚C and the day after experiment the brains and adrenal glands were removed

and weighted. The harvested brains were fixed in the same fixative. Paraffin embedded sections of 10 µm thickness were cut on microtome. The sections were selected according to the Systematic Random Sampling (SRS) and stained with H and E. The 50 fields (5 sections and 10 fields for each section) for each animal were selected randomly and the number of astrocytes were counted according to the previous study. The surface of the HB were measured by Olympus microscope equipped with CellSens software.

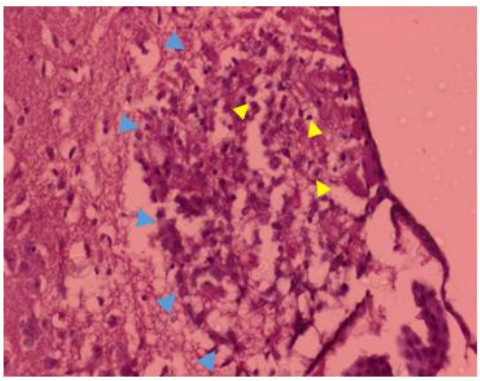
Statistics

All data are expressed as mean ± SD, statistical comparison between two groups was made using student t-test. Statistically significant difference was accepted at the $p<0.05$ level.

RESULTS

The most striking microscopic features of the HB in ketamine exposed animals were hyperchromatic and compacted neurons with disrupted structure (Figure 1).

Figure 1. The microscopic view of extracellular matrix of Habenula (HB) in ketamine exposed animals.



The Extracellular Matrix (ECM) showed disruption and vacuolization in the ketamine exposed animals. Additionally, in some samples presence of numerous astrocytes in vicinity of healthy appearing neurons were noticeable. In control animals the structure HB showed no disruption and vacuolization. Histomorphometry the HB in the experimental animals showed no meaningful difference with those of the control animals ($p>0.05$). The counting of the number of astrocytes and dark neurons was performed according to modified stereological method.

The number of counted astrocytes in experimental group showed significant difference in comparison to those of the control group ($P<0.05$). Additionally, the number of dark neurons in the medial HB of experimental group (15 ± 4) that showed significant difference in comparison with those of control (dead neuron were not seen) ($p<0.05$). The weight of the unilateral adrenal gland was measured. The wet weight of the adrenal gland in the experimental group [42.5 ± 7 mg] showed significant level of difference in comparison with those of control [18 ± 1.20 mg] ($P<0.05$).

The weight of wet brain (the brain stem and cerebrum were removed) in ketamine group [1.28 ± 0.04 gr] showed meaningful difference in comparison with control [1.71 ± 0.2 gr] ($P<0.05$).

DISCUSSION

The HB is a phylogenetically old brain structure and is divided into the Medial Hb (MHb) and the Lateral Hb (LHb). Recent findings indicate that the HB plays a prominent part in such behavioral choice through its effects on neuromodulator system. Additionally, the HB is involved behavioral responses to pain, stress, anxiety, sleep and

reward, and its dysfunction is associated with depression and schizophrenia. The results of this study revealed that chronic exposure to ketamine is not associated with decrease in surface area of the HB. Furthermore, our results showed that chronic use of ketamine leads to reactive gliosis and neuronal death in the medial HB. As a noncompetitive NMDA receptor antagonist, ketamine, is widely used in medicine. Although ketamine use and its effects on HB has been noticed in treatment of depression, a growing body of evidence suggests that ketamine may cause neuronal damage in several major brain regions in rodents and nonhuman primates. Hayashi et al reported that repeated ketamine exposure correlates with increased neuronal degeneration in the developing rat brain. In another study Jin et al showed that ketamine induces tau hyperphosphorylation at serine 404 in the brain areas involved in memory. The results of this study are in line with the previous reports and suggest the neurotoxic effects of chronic ketamine use on nervous tissue. *In vitro* study showed that ketamine leads to increase in the generation of Reactive Oxygen Species (ROS) and Ca^{2+} influx. Therefore, prolonged ketamine use could be considered as an exogenous stressor that may interrupt Hypothalamo-Pituitary-Adrenal (HPA) axis which in turn increases plasma level of corton. In our study the adrenal gland of ketamine exposed animals showed a massive hypertrophy that may reflect HPA over activity due to increased levels of stress. Increased level of oxidative stress in turn promotes astrocytes activation. Astrocytes are heterogeneous and pleomorphic macroglial cells population in the Central Nervous System (CNS). They play paramount roles in neurodevelopment, energy metabolism, vascular coupling in the CNS, antioxidant production and ECM synthesis. It is well-established that one of the fundamental functions of astrocytes is to uptake synaptic-released glutamate, which maintain neuronal functions and prevents glutamate excitotoxicity. Ketamine is known as a competitive NMADA receptor antagonist, but its use leads to increased level glutamate in presynaptic zone. The main route of glutamate uptake is achieved through two types of glutamate transporters, Na^{+}-independent and Na^{+}-dependent transporters. Interestingly some isoform of Na^{+}-dependent transporters is mainly expressed by astrocytes. Ergo astrocytes have the ability to maintain glutamate homeostasis, support normal neuronal function, and protect against glutamate excitotoxicity. Increased number of astrocytes, reactive gliosis, in the HB may reflect the perturbed neuronal homeostasis resulted from ketamine exposure. Another set of our findings showed that chronic exposure to ketamine is associated with decreased brain's weight. A possible explanation to this finding could be the decreased grey matter volume although other likely mechanisms such as brain water content should not be eliminated. On the other hand, histomorphometry of the HB could not reveal significant change in the surface area of ketamine exposed animals. Even though volumetric changes of the HB has been noticed in psychiatric disorders such as schizophrenia, recent studies failed to provide solid evidence on the HB volumetric changes in schizophrenia. Admittedly the comparison between the results our study and the reports on the volume of HB in schizophrenic cases could be naïve and any conclusion should be taken cautiously. Another explanation for the results of histomorphometry could be related to time factor. In another word volumetric or histomorphometric alterations may present a chronologic pattern.

CONCLUSION

In conclusion the effects of chronic exposure to the ketamine primarily leads to neuroglial reaction as gliosis and dark neurons formation. These sets of findings are likely associated with ECM changes. Due to some limitations, we could not perform specific staining method to detect the changes of the ECM, but it is highly recommended to take advantages of ultra-structural and immunohistochemistry methods in order to reveal the possible changes at synaptic level.

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