THE MEDIAN RAPHE NUCLEUS LESION ATTENUATES AMNESIC EFFECTS OF KETAMINE ON SHORT-TERM MEMORY IN RATS

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SUMMARY: The effects of pre-anesthetic dose of ketamine (10 mg per kg) on acquisition of short-term memory in rats with damage of mesolimbic serotonergic system has been investigated. Chemical lesion of serotonergic neurons in the median raphe nucleus was performed by ibotenic acid. The rats injected ketamine (10 mg per kg i. p.) or vehicle (1 ml per kg i. p.) prior to learning were tested after changes in locomotor activity disappeared. Short-term memory was studied using a two compartment single trial passive avoidance chamber for 180 s. In rats with lesion of median raphe nucleus, the pre-anesthetic dose of ketamine produced a significant decrease reduction of acquisition latencies and percentage of animals without amnesia syndrome compared to naive and sham-operated animals-treated with ketamine. These result suggest that deficits in serotonin release of mesolimbic system attenuates amnesic effects of ketamine on short-term memory.

Key Words: Ketamine, short-term memory, serotonin, median raphe nucleus lesion.

INTRODUCTION

Ketamine hydrochloride, a phenylcyclohexylamine, produces a rapidly acting analgesia and anesthesia in both humans and animals, but has adverse effects such as severe disorientation, alterations of mood, retrograde and anterograde amnesia in postoperative period (1,2,3). Because of high incidence and severity of psychologic side effects, neurophysiological basis of amnesic effects ketamine was studied by a number of investigators to reduce the innate behavioral toxicity of the drug.

It might be predicted that ketamine would have amnesic effects via N-methyl-D-Aspartate (NMDA) type of excitatory amino acid receptors that are located in the hippocampal structure, a brain region thought to be important in memory process (4,5). However, ketamine also inhibits neuronal reuptake of serotonin (5-HT) in synaptic cleft and increases brain 5-HT turnover (6,7). Among many other functions, 5-HT neurotransmission plays an important role in mechanisms of learning and memory (8,9). Hence, assessment of whether or not and to what extent the brain 5-HT is functional on ketamine-induced memory decay is important for identification of mechanisms potentially responsible for postoperative complications following ketamine anesthesia.

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In the present study, we examined the character of amnesia caused by pre-anesthetic dose of ketamine on short-term memory trace in the rats with lesions of median raphe nucleus.

MATERIALS AND METHODS

Male Wistar rats weighing 200-250 g were used, with free access to food pellets and tap water. A 12:12 h light/dark cycle was maintained (light on 07 am).

Surgical procedures

The rats in sham and MnR-lesioned groups were anaesthetized with sodium pentobarbital (50 mg/kg/body weight i. p.). They were then placed in a stereotaxic instrument (Stoelting) and injection cannula (24 gauge stainless steel tubing) to be positioned into the median raphe nucleus at stereotaxic coordinates (from bregma: 6900-7200 μm, L: 00.00; 8000 μm deeper from skull surface) according to atlas of Konig and Klippel (1963). Neurotoxin ibotenic acid (Sigma I2765), for chemical damage of median raphe nucleus, that dissolved in artificial cerebrospinal fluid without glucose (Locke's solution), at a dose of 2-2.5 μg in 0.3-0.5 μl total volume was injected slowly using a micro-pump (Stoelting, model 210) during 20 min. The sham operations underwent similar procedures, except 0.3 μl Locke's solution was administrated into the MnR region instead of ibotenic acid. All rats were allowed to recovery for ten days following these surgical procedures.

Drug administration

The drug used in this study was ketamine hydrochloride (Parke-Davis, 50 mg ml^{-1}) as a commercial preparation and then further diluted with saline (0.9% NaCI) to the appropriate concentration. Experiment was performed with rats divided into two groups. Prior to learning, the one group of naive, sham-operated and MnR-lesioned rats were treated with ketamine (10 mg/kg body weight) and second group of naive, sham-operated and MnR-lesioned rats received 0.9% NaCI (1 ml/kg body weight) by intraperitoneal route. After disappearance of changes in locomotor activity, experiments were performed.

Behavioral procedure

Short-term memory was studied using passive avoidance paradigm that consists of two compartments as an illuminated 'safe' and a dark 'punishable' one. In the acquisition trial, rat was placed in an illuminated box (25x17x17 cm in size). Once the rats prompted by their instinct stepped its four paws into the dark compartment (27x27x27 cm in size), the slide door was lowered a 1,5 mA foot shock was delivered for 3 s through the wire mesh floor to its paws. After the foot-shock applied, the rats were immediately taken out from the dark compartment and returned to the illuminated one. At the same

Figure 1: Schematic presentation of the median raphe nucleus and neighboring structures according to atlas of Konig and Klippel (1963). Abbreviation used in figure : DR=Dorsal raphe nucleus; ml = Medial lemniscus; CP= Cerebral pedinculi; NP = Pontine nuclei.

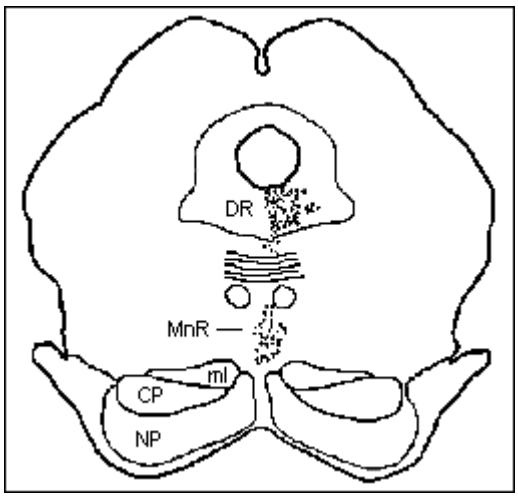
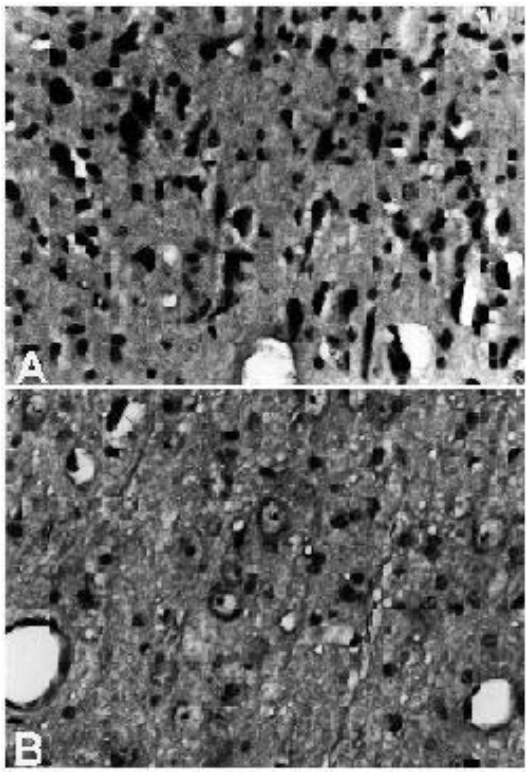


Table 1: Effects of ketamine on median entery and acquisition latencies in naive, sham-operated and MnR-lesioned rats.

	Median Latencies (s)		Interquatile range	
	Entery	Acquisiton	Q1	Q2
Vehicle-treated				
Naive (n=10)	27.5	180	180	180
Sham-operated (n=10)	17	180	180	180
MnR-Lesion (n=10)	15.5	180	180	180
Ketamine (100 mg kg)				
Naive (n=6)	21	23*	23	170
Sham-operated (n=7)	20	24*	11	180
MnR-Lesion (n=9)	12	180**♦	76	180

* p<0.001 (Compared to vehicle-treated normal rats.)
** p<0.05 (Compared to vehicle-treated MhR lesioned rats.)
♦ p<0.05 (Compared to normal rats.) (naive and sham-operated)-receiving ketamine at dose of 10 mg per kg
Groups were compared with Mann-Whitney U test.

Figure 2: Histological brain sections taken at level of median raphe nucleus A: Control animal receiving Locke's solution alone into the MnR, B: MnR-lesioned animals treated with ibotenic acid. Neurons in MnR appear to be intact in control rats, severe cell body loss seen in MnR-lesioned rats (x800).



time, the slide door was opened and the acquisition test was given for 180 s. The latencies defined as the time it took an animal to completely enter the dark compartment. Entery latency prior to learning and acquisition latency immediately after learning were recorded. In each group, the percentage of animals avoiding dark compartment for 180 s (animals without amnesia syndrome) was calculated.

Histology

To determine the sites of lesion, at the end of the experiments, all animals were deeply anesthetized with pentobarbital sodium (60 mg/kg/body weight). The brains were perfused transcardially with isotonic saline (0.9% NaCI) followed by formal in (10%) in phosphate-buffered saline (pH 7.4). The brains were then removed and stored in the same solution, to be later sectioned in the coronal plane into 5 μm thickness with a micro-tome (reichert). The brain sections were stained with Hematoxylin-Eosin. The position and site of damage induced by ibotenic acid was determined by examining these sections under the light microscope and identified in accordance with the atlas of Konig and Klippel (1963).

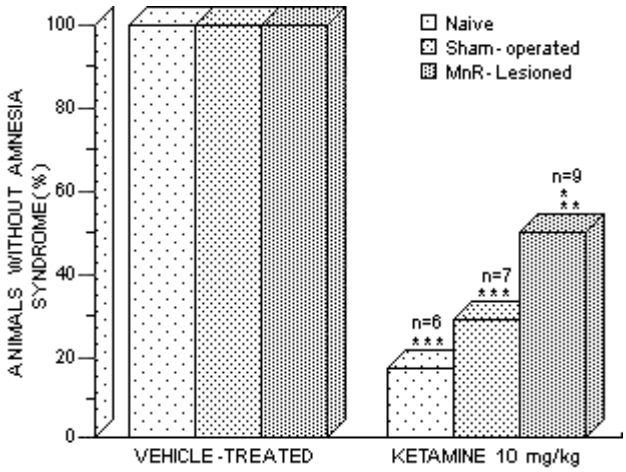
Statistics

Median latencies (entery and acquisition) for vehicle-and drug-treated groups were analyzed with Kruskal-Wallis test. Individual comparison between groups were analyzed using Mann Whitney U-test. The percentage of animals without amnesia syndrome between 2 groups was compared by using x^2 test. Probability level of 0.05 or less was accepted as a significant difference.

RESULTS

A 10 mg per kg dose of ketamine produced a mild excitation with occasional head movements (head swinging), body rolling (side to side movements of the hind quarters) and hyper-locomotion in the normal and MnR-lesioned rats (latency of about 1-2 min; duration

Figure 3: The percentage of animals without amnesia syndrome during acquisition test for 180s (analyzed with x^2 test). *p< 0.05 (significantly different from ketamine administrated normal rats). **p<0.05 (compared to vehicle-treated MnR-lesioned rats.) ***p<0.001 (compared to vehicle-treated normal rats). Data base on 10 rats for each vehicle-treated group.



of action about 15 min). The ketamine treated animals appeared normal at the end of the 15 minutes as vehicle treated-control rats.

Histological analysis of brain slices showed ibotenic acid induced large neuron cell body loss (polygonal and bag-like) in the MnR region (Figures 1 and 2).

Analysis of median entry latencies prior to learning, considered as an inborn illumination escape reveal no differences between vehicle-and ketamine (10 mg per kg)-treated naive, sham-operated and MnR-lesioned rats (Table 1).

In naive, sham-operated and MnR-lesioned groups receiving the vehicle, the median acquisition latencies was 180 s, equal to the maximum possible latency of acquisition test, and all animals avoided the dark compartment (Figure 3). In contrast, normal rats (naive and sham-operated) given ketamine at doses of 10 mg per kg displayed a significant decrease in acquisition latencies ($p<0.001$, Table 1), and a significantly small percentage of animals without amnesia syndrome (naive 16.6 %, sham-operated 28.5 %) compared to vehicle-treated control rats (naive 100%, sham operated 100%) ($p<0.001$; Figure 3).

The MnR-lesioned rats given ketamine had median acquisition latencies of 180 s and percentage of animals without amnesia was 50% during acquisition test, significantly different from vehicle-treated MnR-lesioned rats ($p<0.05$). The rats with MnR lesions treated with ketamine exhibited a significant decrease in ketamine-induced acquisition deficit (Table 1), and a much higher percentage of animals without amnesia syndrome compared to ketamine-treated normal rats ($p<0.05$) (Figure 3). In ketamine administered rats, vocalizations and jumping were noted when foot shock occurred, thus precluding analgesia and anesthesia as a cause of acquisition deficits, much as in the vehicle treated rats.

DISCUSSION

The main result of present study was that the lesion of median raphe nucleus produced by ibotenic acid attenuates amnesic effects of ketamine on short-term avoidance memory.

Recent reports suggest that the effects of ketamine on passive avoidance response are mediated through its activity at the NMDA receptor that are concentrated in the hippocampus (10,11). It has also been shown that hippocampal NMDA receptors are related to acqui-

sition memory (12). The NMDA antagonist 2-amino-5-phosphonodentanoic acid (AP5) given intra- cerebroventricularly prior to acquisition of passive avoidance response results in a disruption of retention performance (11). Similar results were found when AP5 was administrated directly into the hippocampus (13). In our experiments, in normal rats, ketamine produces a decrease in acquisition latencies of a passive avoidance response in agreement with previous reports.

Ketamine also increases 5-HT content and turnover in the brain (6,7). It has been determined that the median raphe nucleus (MnR) region of brain stem characterized by 5-HT-ergic principal cells (polygonal and bag-like) that establish prominent connections with septohippocampal formation considered as a part of the mesolimbic system (14-16). Histochemical experiments revealed that neurotoxin ibotenic acid induced principal cell body loss of median raphe nucleus results in a decrease in hippocampal serotonin level in the rats (17). While under the influence of ketamine, MnR-lesioned rats displayed a significantly longer acquisition latencies of passive avoidance compared to identical dose ketamine-treated normal rats. These results indicate that the mesolimbic 5-HT-ergic denervation attenuates ketamine-induced short-term memory decay.

Recently it is reported that blockade of hippocampal 5-HT2 receptor or activation of 5-HT1A auto-receptors resulted in an improvement in acquisition of new information (18,19). It is also shown that augmentation of 5-HT content of hippocampus prior to learning produced an impairment in storage of trace memory (20). Otherwise 5-HT injection into the hippocampus after acquisition for testing permanent trace memory caused a prolongation of passive avoidance response, suggesting that mesolimbic serotonergic system mainly involved in mechanism of retrieval past experience (21,22). It is therefore, possible that the deep amnesic effects of ketamine might be connected with two processes: (1) decaying of acquisition mechanism due to hippocampal NMDA blockade; (2) interference action of spontaneous recalling of past experience induced by increased mesolimbic 5-HT-ergic activity. Perhaps postoperative hallucinations caused by ketamine can be possessed by its action on mesolimbic 5-HT-ergic system. In this regard, in rats with damage of mesolimbic 5-HT-ergic system, ketamine fails to stimulate hippocampal mechanism of spontaneous recalling from permanently stored information, that resulted in attenuation amnesic effects of this drug on acquisition of recent events. Thus, our results allowed to consider that mechanism of amnesic effects of ketamine may be due to inhibition of hippocampal NMDA receptors and increased activity of mesolimbic 5-HT-ergic transmission.

ACKNOWLEDGEMENT

This study was supported by the Scientific and Technical Research Council of Türkiye (TUBITAK) and Çukurova University Research Fund.

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