EFFECTS OF KETAMINE ON MEMORY AND NOCICEPTION IN RATS

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ABSTRACT

BACKGROUND: Ketamine is intravenous anaesthetic with NMDA-glutamate receptors mechanism of action.

MATERIAL AND METHODS: Male Wistar rats were treated with saline (group A) or 10, 15 or 20 mg/kg of ketamine (groups B, C and D, respectively). For active avoidance test an automatic reflex conditioner was used. The observed variables were number of avoidances, escapes and intertrial crossings. Step-through and step-down passive avoidance tests were done with learning and memory retention test. Criteria for step-through test were latency of reactions of 180 sec in the light chamber. Criteria for step-down test were latency of reaction of 60 sec on the platform. The hot-plate test evaluates the reaction time of the rats dropped on a heated surface. The analgesy-meter test exerts a force increased at constant rate.

RESULTS: In active avoidance test the controls increased the number of avoidances during learning and memory tests. Ketamine in all doses used increased the number of avoidances during learning and memory test. Controls did not change the number of escapes, but the ketamine treated animals decreased it. The number of intertrial crossings was not changed by controls or ketamine-treated rats during learning and memory tests. In passive avoidance tests the controls and ketamine-treated rats increased the latency time during learning and memory retention tests. In hot-plate analgesic test and in analgesy-meter test the controls and ketamine-treated rats did not change the latency of reaction.

CONCLUSION: The results suggest that ketamine improves learning and memory processes and has no analgesic effect in the doses applied.

Key words: ketamine, learning, memory, rats

INTRODUCTION

The role of glutamatergic mediation in synaptic transmission and plasticity is not fully understood. Recent research has focused on determining the role of the three main glutamate receptor classes in the processes of learning and memory: N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and the metabotropic glutamate receptors. NMDAs, the receptors involved in memorizing new information, play a major role in glutamatergic transmission. Current evidence for the role of AMPA, the receptors involved in processes of learning and memory, is insufficient. This is due to the fact that block of this receptors switches off communication between neurons that influences many specific learning and memory mechanisms. The AMPA receptors are probably involved in the processes of learning and memory. The role of metabotropic receptors in perception and analysis of new information is not significant. Ketamine is intravenous anaesthetic with NMDA–glutamate receptors mechanism of action. Recent data show that NMDA receptors are also involved in the mechanism of the antinociceptive action of ketamine.

The aim of our study was to evaluate the effects of ketamine on two analgesic tests and on active and passive avoidance tests in rats.

MATERIAL AND METHODS

Male Wistar rats weighing 170-230 g were divided into 4 groups (n = 10). Rats were kept under standard laboratory conditions in a 08:00-20:00 h light/dark
cycle and were provided with food and water *ad libitum*. The drug was administered intraperitoneally 30 min before testing. The following experimental groups were used: group A received saline (0.1 ml/100 g b.w.); group B - 10 mg/kg of ketamine; group C - 15 mg/kg; and group D - 20 mg/kg of ketamine (calypsol, amp. 50 mg/ml-10 ml, Gedeon Richter, Hungary).

**Behavioural tests**

The active avoidance test was performed in a shuttle box. An automatic reflex conditioner was used (Ugo Basile, Italy). Learning session was held for 5 consecutive days and consisted of 30 trials (6 sec light and buzzer, 670 Hz and 70 dB, followed within 3 sec by random 0.4 mA foot electrical stimulation and a 12 sec pause). Seven days later a 1-day memory retention test was performed using the same parameters without foot stimulation.

The following behavioural signs were observed:

a) number of correct responses on conditioned stimuli, i.e., avoidances; b) number of escapes from foot stimulation (unconditioned stimuli responses); c) number of intertrial crossings.

Two passive avoidance tests were used: step-through and step-down.

The step-through passive avoidance test was performed in an automatic set-up 2 compartments cage (Ugo Basile). Learning and retention sessions consisted of three trials (door delay 7 sec, followed by electrical stimulation for 9 sec at an intensity of 0.4 mA). The latency of reactions (the animal remaining in the light chamber for more than 180 sec) was used as criterion for learning and retention.

The step-down passive avoidance test was performed in a set-up 1 compartment cage with a plastic platform (Ugo Basile). Learning and retention sessions consisted of two trails (electrical stimulation duration of 10 sec with intensity of 0.4 mA). The latency of reactions (the rat remaining on the platform for more than 60 sec) was accepted as criterion for learning and retention.

**Nociceptive tests**

**Hot-plate test**

A transparent glass cylinder was used to keep the rat on the heated surface of the plate (Ugo Basile). The temperature of the hot plate was set to 55 ± 0.5 C. Time of latency was defined as the time period between the zero point when the animal was placed on the hot plate surface and the time when the animal licked its back paw or jumped off to avoid thermal pain. The accepted "zero time" of this study starts 30 min after the drug injection. To minimize tissue damage, a cut-off time of 60 sec was adopted. The latencies of both forepaw licking or jumping were measured for each animal at 0 and 60 min.

**Nociceptive test**

The antinociceptive effect of ketamine was assessed using a mechanical noxious stimulus as previously described by Randall & Selitto. Nociceptive threshold, expressed in grams (g), were measured with an algolivermeter (Ugo Basile) by applying pressure to the right hind paw of unrestrained rats until a squeak and/or a struggle was obtained (a cut-off level of 300 g was applied). The rats were tested on the first and second hour after i. p. administration.

**Statistical analysis**

The obtained values were expressed as mean ± SEM. The comparison between the groups was made by Student’s t-test of analysis of variance (one way ANOVA), in the INSTAT computer program. A value of P < 0.05 was considered as a significant difference. A two-way ANOVA for GLM repeated measurements was used to compare different groups with the respective controls for learning and memory tests.

**RESULTS**

In the active avoidance test the control rats showed a significantly increased number of conditioned stimuli responses, i. e., avoidances on 3, 4 and 5 days (P < 0.05) on learning session as well as on memory retention test (P < 0.05) compared to day 1. Rats treated with ketamine in all doses showed an increased number of avoidances on 4 and 5 days of learning session (P < 0.05) as well as on memory retention test (P < 0.05), compared to the first day of respective dose (Fig. 1).

Control rats did not change the number of unconditioned stimuli responses (escapes) during the learning session or during the memory retention test compared with that of day 1. Rats treated with 10 mg/kg or 15 mg/kg of ketamine did not change the number of escapes either during the all learning session or on memory retention test. Ketamine in the highest dose (20 mg/kg) used decreased the number of escapes (P < 0.05) used on learning and memory tests, compared to the day 1 control (Fig. 2).

The control group showed a decrease number of intertrial crossing on 4 and 5 days (P < 0.05) of learning session compared to day 1. Rats treated
with 20 mg/kg of ketamine showed a decrease of the number of intertrial crossing on day 1 (P < 0.05) of learning session compared to the same day control (Fig. 3).

In passive avoidance test step-through the controls increased the latency time (P < 0.05) during learning, short and long memory retention tests, compared to day 1 control. Ketamine-treated rats (20 mg/kg) prolonged the latency on day 1 (P < 0.05) compared to the control. Ketamine treated rats at all doses applied prolonged the latency (P < 0.05) on day 2 of learning, short and long memory retention tests compared to day 1 of respective dose (Fig. 4).

In passive avoidance step-down test, control rats increased the latency time (p < 0.05) on day 0 - P < 0.05 compared to the first day control; * - P < 0.05 compared to the first day of respective dose.

**Figure 1.** Effects of ketamine on number of avoidances in active avoidance test.

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<thead>
<tr>
<th>Days of testings</th>
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<td>sal</td>
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* - P < 0.05 compared to day 1 of control.

**Figure 2.** Effects of ketamine on number of escapes in active avoidance tests.
2 of learning session, short and long memory retention tests compared to day 1. Rats receiving ketamine at doses of 15 mg/kg and 20 mg/kg showed increased latency of reaction times ($p < 0.05$) during day 2 of learning tests, short and long memory retention tests compared to day 1 of respective dose (Fig. 5).

In hot-plate analgesic test the controls and ketamine-treated rats (at all doses applied) did not changed significantly the latency of reaction (Fig. 6).

In analgesy-meter test the controls and ketamine-
treated rats (at all doses applied) did not change significantly the latency of reaction (Fig. 7).

DISCUSSION

It is well known that two main brain structures – the cerebral cortex and hippocampus - play role in processes of learning and memory and glutamate mediation is involved in them.\(^4\) There are data that non-competitive NMDA-receptor antagonist ketamine across a broad dose range (5-30 mg/kg) change social behaviour in mice without affecting any learning processes. Gao et al.\(^5\) suggest that medial temporal lobe of the cortex has been implicated. Other researchers\(^6\) establish antidepressive effect of ketamine and suggest that NMDA-receptors in hippocampus are involved in that process.

Our results showed that the intravenous anaesthetic ketamine, used at subnarcotic doses improved
learning and memory processes, especially in active avoidance test. Probably ketamine play role in process of forming long-term memory in active avoidance test as well as in passive avoidance test. There have been reports that acute treatment with ketamine increases glucose utilization in the rat prefrontal cortex. This data support our findings for improvement of learning and memory by a short ketamine treatment, due probably to the enhanced metabolic processes in this brain region. In addition, subchronic ketamine treatment reduces both mesocortical dopamine utilization and markers of GABA-ergic interneurons in prefrontal cortex and hippocampus. There are also some contradictory data with other NMDA receptor antagonist phencyclidine in mice performing a Morris water maze task or on working memory on a radial-arm maze task in rats and in mice. One possible explanation for these discrepant findings may be related to differences in drug treatment and behavioural testing protocols. Another explanation for the inconsistent effects of these treatments on working memory may be related to the specific type of task used to assess these functions. An important consideration in evaluating our results is that our study was designed to investigate the effects of ketamine on acquisition and/or consolidation in active and in passive avoidance tests and rats did not receive any trainings prior to drug treatment. Therefore we claim that we evaluate a pure drug effect. Other authors trained the rats before treatment until they achieved the criterion in behaviour test used prior to ketamine exposure.

It is known that NMDA glutamate receptors are involved in the development of proprioceptive sensibility at spinal level and that ketamine as selective NMDA-receptor antagonist affects the neuropathic pain. Other researchers showed that ketamine also affects the opioid transmission in the brain. Furthermore, Hirota et al. found the interaction of ketamine with mu, kappa and delta opioid receptors, applied at doses producing deep anaesthesia.

Our findings show that ketamine administered at doses of 10, 15 or 20 mg/kg doesn’t affect significantly the pain threshold. New data by Engin et al. suggest an anxyolytic and antidepressant-like properties of ketamine using some behavioural models. Probably in our study subnarcotic doses of ketamine did not exert any anxyolitic or antidepressant-like effect.

In conclusion, our findings indicate that ketamine influenced learning and memory processes in active and passive avoidance tests and did not affect nociception and pain threshold in rats.

REFERENCES
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Effects of Ketamine on Memory and Nociception in Rats


