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Ketamine also decreases serum BDNF levels and spatial learning while causes apoptosis in neonatal rats

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Keypoints

Ketamine which is one of most used anesthetic in pediatric population has adverse effect on the spatial learning in the neonatal rats. This adverse effect may depends on reduced serum BDNF levels and/or increased apoptosis.

Abstract

Introduction

The aim of this study were investigate the effects of early and late of ketamine in the neonatal rats.

Material and methods

The study included a total of 24 seven-day-old male Wistar-Albino rats. Subjects were randomly divided into two groups: Group C and Group K. In Group K, anesthesia was achieved with intraperitoneal administration of 4 doses of 20 mg/kg ketamine in 90 minute intervals. Then, half of the subjects were sacrificed to determine the early effects of anesthetics (Group KE, Group CE), while the rest of the subjects were sacrificed 6 weeks after the application to determine the late effects of anesthesia (Group KL, Group CL). Serum BDNF levels were measured by ELISA; brain BDNF and caspase-3 levels were assessed immunohistochemically. The behavior, anxiety states and spatial learning abilities of the subjects during the long-term period were evaluated by using the plus arm test and the Morris water test, respectively.

Results

Serum BDNF levels had significantly lower in Group KE ($p<0.05$). BDNF levels in the cerebral cortex and hippocampus was no significant difference between groups. There was a significant negative correlation between serum and cortex BDNF levels ($p=0.01$, $r=-0.601$). Cortex caspase-3 levels were significantly higher in Groups KE than Group CE ($p<0.05$). There was no difference between groups in terms of open arm index, locomotor activity and Morris water tests. When the platform was removed, the time spent in the quadrant was significantly shorter Group KL ($p<0.05$).

Conlcusions

Ketamine effect on spatial learning maybe by reducing the serum BDNF levels and/or increasing apoptosis.

Keywords

anesthesia, apoptosis, brain derived neurotrophic factor, ketamine, learning

Introduction

General anesthesia is considered safe, experimental studies have shown that it might have harmful effects on the developing mammalian brain. Ketamine, a NMDA receptor antagonist, is widely used in pediatric patients due to its analgesic properties. In recent studies, it has been demonstrated that repeated administration of ketamine and other NMDAR antagonists increased neuroapoptosis in the developing brains of neonatal rats (1,2).

Brain-derived neurotrophic factor (BDNF) has important implications in the survival, growth and differentiation of existing neurons in the central and peripheral nervous system (3,4). At the same time, BDNF was shown to be active in the hippocampus, cerebral cortex, cerebellum and basal forebrain which are the areas that carry out vital functions such as learning, memory and thinking (5).

The aim of this study were to investigate the early and late effects of ketamine on the neonatal rat brain.

Material and Methods

After receiving approval from the University's Animal Experimentation Ethics Committee, subjects were obtained from the University Experimental Research Center and the study was initiated at the same center. The Helsinki Universal Declaration of Animal Rights was adapted at every stage of the study. The study included a total of 24 seven-day-old male Wistar-Albino rats. Rats were kept in the rooms with ambient temperature of 22-24 °C and with 12/12 hour day/night cycle. Except for the time it took for the experimental tests, subjects were kept in the same cage with their mothers until postnatal day 21. After the 21st day rats were put in separate cages and fed with standard rat chow and tap water. Subjects were randomly divided into two groups: Group C (control, n=12) and Group K (ketamine, n=12). In Group K, anesthesia was achieved with intraperitoneal administration of 4 doses of 20 mg/kg ketamine in 90 minute intervals. The concentration of anesthetic was adjusted according to the tail test. The tail test was applicated every 15 minutes. When middle 1/3 of the tail was clamped, if there was response, ketamine was administered as additional dose about 15% of doses given every 90 minutes. All subjects were put in a plastic, transparent anesthesia chamber that was connected to an anesthesia device and was ventilated with 4L/min flow and 50% O2-air mixture. At the end of the application period, half of the subjects were sacrificed to determine the early effects of anesthetics (Group KE, Group CE), while the rest of the subjects were sacrificed 6 weeks after the application of to determine the late effects of anesthesia (Group KL, Group CL). Serum BDNF levels were measured by the ELISA method (EK0308, Boster Biological Technology, Ltd.). The BDNF levels (Abcam, ab108319, Cambridge, UK) and caspase-3 levels (Abcam, ab13847, Cambridge, UK) were assessed immunohistochemically in other brain hemispheres (0: no staining, 1: mild, 2: moderate, 3: severe). the ELISA method; brain BDNF levels and caspase-3 levels were assessed immunohistochemically. The behavior, anxiety states and spatial learning abilities of the subjects during the long-term period (6 weeks later) were evaluated by using the plus arm test and the Morris water test, respectively.

Results

Serum BDNF levels had significantly lower in Group KE ($p<0.01$). BDNF levels in Group CL and Group KL were higher than in Group CE and Group KE ($p=0.001$) (Figure 1). BDNF levels in the cerebral cortex and hippocampus was no significant difference between groups. However, the cortex BDNF levels were decreased over time in Groups C and K ($p<0.05$) (Figure 2). There was a significant negative correlation between serum BDNF levels and the cortex BDNF levels ($p=0.01$, $r=-0.601$). In the early period cortex caspase-3 levels were significantly higher in Groups KE than in Group CE ($p<0.05$) (Figure 3). However, there was no significant difference in the level of hippocampal caspase-3 in any of the groups in the early period. When early and late periods were compared, caspase-3 levels were found to decrease over time; however this reduction was not significant.

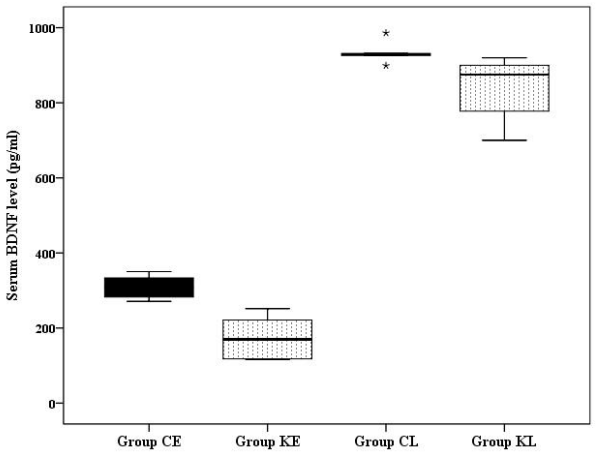

Figure 1. Serum BDNF levels

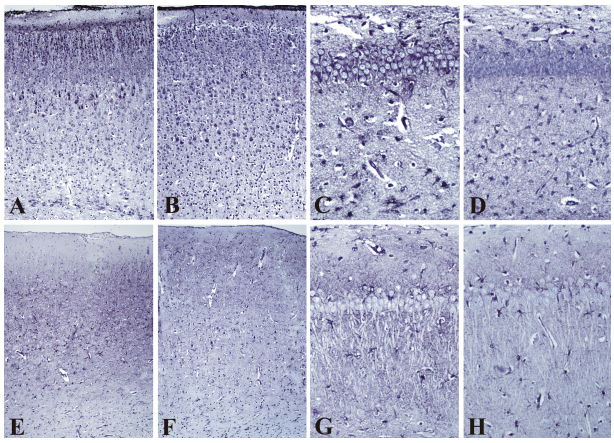

Figure 2. BDNF immunohistochemistry staining in the corteks (200µm) (A: Group CE, B: Group KE, E: Group CL, F: Group KL); in the hipocampus (C: Group CE, D: Group KE, G: Group CL, H: Group KL)

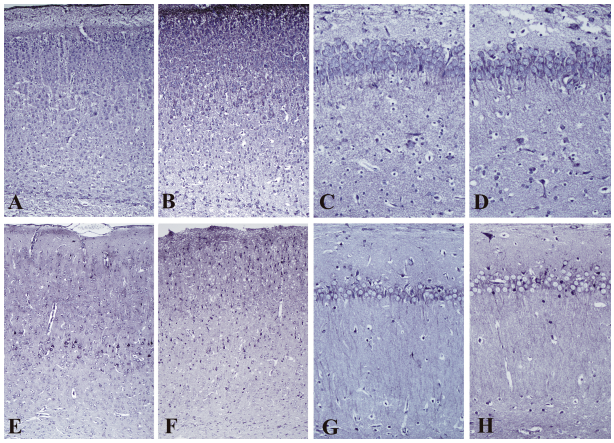

Figure 2. Caspas-3 immunohistochemistry staining in the corteks (200µm) (A: Group CE, B: Group KE, E: Group CL, F: Group KL); in the hipocampus (C: Group CE, D: Group KE, G: Group CL, H: Group KL)

There was no significant difference between groups in terms of open arm index, locomotor activity. Morris water tests showed no significant difference between groups. It was observed that in both groups the time to reach the platform became shorter with time ($p<0.05$). When the platform was removed, the time spent in the quadrant where the platform used to be was significantly shorter Group K compared to Group C ($p<0.05$).

Discussion

Ketamine, a noncompetitive NMDA receptor antagonist, produces a unique dissociative anesthetic state, which is particularly useful for inducing anesthesia in children for surgical procedures inside or outside the operating room (6). Serum BDNF levels are known to increase with age (7). In our study, we found that serum BDNF levels were significantly higher in the seven-week rat compared to neonatal rats. We observed serum BDNF levels decreased, while brain BDNF levels didn't change in Group KE. Ibla et al. (8) have demonstrated that prolonged exposure to ketamine increased BDNF levels in developing rat brains. They have found neurodegeneration, BDNF and TrkB cDNA products and protein levels had increased in ketamine-treated neonatal rat. Garcia et al. have explained while low doses of ketamine did not result in changes in hippocampal BDNF levels, higher doses were shown to increase them (9). Another study has also demonstrated that pro-apoptotic anaesthetic drugs modulate BDNF protein levels in the developing brain (10). It was thougt that these differences related to different doses and durations of used ketamine.

In studies with seven-day-old rats where loss of pyramidal neurons was induced, levels of caspase-3, caspase-9 and apoptotic neurodegeneration were increased in the hippocampus upon administration of ketamine (11-15). In our study, while cortex caspase-3 levels were significantly higher in the ketamine group compared to the control group, hippocampal levels were not.

In our study, we found insignificant difference between groups in terms of open arm index, locomotor activity

and the time to reach the platform in Morris water test. But, when the platform was removed, the time spent in the quadrant where the platform was significantly shorter ketamine groups. Wang et al. have demonstrated that ketamine (80 mg/kg) induced the learning and memory impairment and neurodegeneration, while the repeated low dose ketamine (30 mg/kg) did not impair the learning and memory function in adolescent rats (16). Some studies have showed that ketamine administration in 30 mg/kg for long periods altered behavioral change in rats (17,18), we thought the methodology used might account for the difference.

In our study, we observed that ketamine which is one of most used anesthetic in pediatric population has adverse effect on the spatial learning in the neonatal rats. This adverse effect may depends on reduced serum BDNF levels and/or increased apoptosis.

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Conflict of interest: none
Ethical approval: Firat University's Animal Experimentation Ethics Committee (29.12.2011/11/136). Universal Declaration of Animal Rights was adapted at every stage of the study.

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