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Ameliorating effects of low-dose ketamine administrations on opioid-induced memory impairments and neurodegeneration in mice

Emre Uyar[a,*], Ugur Seker[b], Onural Ozhan[c], Muhammet Burak Acikgul[a], Mehmet Colak[c], Sevde Feyza Izci[a], Hakan Parlakpinar[c]

[a] *Inonu University, Faculty of Pharmacy, Department of Pharmacology, Malatya, Türkiye*
[b] *Artuklu University, Faculty of Medicine, Department of Histology and Embryology, Mardin, Türkiye*
[c] *Inonu University, Faculty of Medicine, Department of Pharmacology, Malatya, Türkiye*

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Abstract

Aim: Opioids have indispensable roles in pain management. A strong link exists between opioid use and memory impairments, mainly with continuous use. This study investigated the effects of two opioid drugs, meperidine and fentanyl, on emotional memory functions, brain morphology, and the possible protective effects of low-dose ketamine in mice.

Materials and Methods: A passive avoidance (PA) test was used to measure emotional memory functions following seven daily drug applications in 48 male Balb/C mice (30-35 g). Meperidine (10 mg/kg), fentanyl (0.3 mg/kg), ketamine (5 mg/kg), and combinations of ketamine with the opioids were intraperitoneally injected daily. No drugs were utilized during the testing days. Brain tissues were obtained after sacrification and put into diluted formalin solution for histopathological analysis.

Results: Transfer latencies of the meperidine and fentanyl-treated groups in the PA test were lower than in the vehicle-treated group ($p<0.01$, $p<0.05$, respectively). Ketamine combined with meperidine had higher latencies than in the meperidine-treated group ($p<0.05$). The augmenting effects of ketamine were evident against fentanyl and meperidine-induced neurotoxicity as morphologic alterations were reduced.

Conclusion: Low-dose ketamine may fend against opioid-induced neurotoxicity and emotional memory impairments, especially against meperidine, which can be a practical alternative to fentanyl in clinical settings.

Introduction

Opioids have potent analgesic effects and are indispensable tools in pain management. Beyond the analgesic properties, they have an intricate relationship with cognitive functions, particularly memory [1]. Their effects on memory functions have gained attention in neuroscience, algology, and pharmacology.

Opioids interact with endogenous opioid receptors, influencing pain perception, mood, reward, and various cognitive processes, including memory formation, consolidation, and retrieval [2]. They can influence emotional memory processing, dampening the intensity of memories, making them less vivid, or disrupting the formation of associations between emotions and events [3]. Higher doses and extended applications of opioids can impair memory by affecting neurotransmitter systems involved in memory processes. The impact on neurotransmitters like dopamine, norepinephrine, and serotonin can disrupt the normal functioning of brain regions responsible for memory [4].

Ketamine is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist drug. It is reported to reduce opioid tolerance and requirement and opioid-induced hyperalgesia in sub-analgesic doses [5]. Ketamine is also an activator of multiple neurotrophic signaling cascades with neuroprotective actions, specifically in low doses [6,7]. On the other hand, the involvement of NMDA receptors in opioid-induced cognitive disruptions is reported in various studies [8,9]. Thus, ketamine affecting NMDA receptors can potentially affect opioid-induced neurocognitive disruptions.

This study aimed to investigate the effects of two opioid drugs, meperidine and fentanyl, on emotional memory functions and brain morphology and the possible protec-

*Corresponding author:
Email address: eczemreuyar@hotmail.com (Emre Uyar)

tive effects of ketamine in mice. The morphologic appearances of several brain regions, including the hippocampus, were histopathologically investigated.

Materials and Methods

Animals and drugs

Forty-eight Balb/C mice weighing 35-40 g were separated into 6 groups and put into plastic cages (n=8). The mice were transferred to the laboratory a week before behavioral tests for habituation and kept in standard laboratory conditions (21±1.5°C, 60% relative humidity, 12 h light/dark cycle: light on at 8.00 pm). Food and water were provided ad libitum. They were naive to the conducted test and only tested once. All experiments were carried out with the approval of the local animal ethics committee (Inonu University Animal Experiments Local Ethics Committee) with the number 2022/1-1. All animal procedures were carried out according to the direction of the European Community Council Directive of 24 November 1986. Racemic ketamine (5 mg/kg, Ketalar, Pfizer, Manhattan, New York City, New York, CAS: 6740-88-1), fentanyl (0.3 mg/kg; Fentanyl-Janssen, Janssen, Olen, Belgium, CAS: 437-38-7), and meperidine (10 mg/kg, Aldolan, Gerot, Vienna, Austria, CAS: 57-42-1) were diluted in saline and injected intraperitoneally (10 ml per kilogram body weight) once daily for seven consecutive days around the exact times of the day (around 3:00 pm). Isotonic 0.9% sodium chloride solution was injected into the vehicle group. Injections were made 10 ml per kilogram of body weight. A passive avoidance (PA) test was performed following drug application protocols. The mice were sacrificed using urethane (1.2 g/kg, Selleck Chemicals, GmbH Mathias Brueggen Strasse, Cologne, Germany, CAS: 51-79-6); brain tissues were isolated and stored in a 10% neutral buffered formaldehyde solution for further histologic analyses. All experiments were performed in a semi-soundproof, dimly-lit room.

Passive avoidance test

Passive avoidance is a commonly used behavioral test to assess emotional memory functions in rodents based on contextual fear conditioning [10]. In this test, mice learn to associate a specific place with an aversive stimulus, a mild foot shock. The apparatus (MAY-APAV214) consisted of two chambers (22×21×22 cm each), one with white-colored walls and enlightened with 2000 lux, and the other with black-colored walls with no light) connected with an automated ground-level door (7x7 cm). The floor was covered with an electrifiable grid floor to deliver a shock (0.25 mA/1 s) to animals' paws. The test lasted two days; on the first day, acquisition and on the second, retention trials were conducted. The mice were individually put in the illumined compartment in the acquisition trial and let acclimate for 60 seconds. Then, the door between the chambers opened. It closed automatically after the mice entered the dark compartment; the animals had an electric shock through their paws. A cut-off time of 300 seconds was used; if the mice did not enter by this time, they were excluded from further experiments. A retention trial was executed with the same procedure (without electric shock) a day following the acquisition trial. Transfer latencies to enter the dark compartment were calculated and interpreted as their memory performance. Higher latencies meant better memory, while lower values meant memory impairments.

Tissue processing and routine staining protocol

Tissue processing and staining were performed as described previously [11]. Formaldehyde-fixed tissue samples were washed under tap water for a day, dehydrated through increasing alcohol series, cleared in xylene, and embedded into paraffin blocks. Five µm thick sections were obtained from paraffin blocks with a rotary microtome on adhesive slides, and the sections were used for microscopic investigations. Tissue sections were stained with hematoxylin and eosin (H&E) for pathological observations, and the samples were kept in hematoxylin for 8 minutes, washed under tap water, and stained in eosin for 3 minutes. Rinsed in alcohol series and cleared in two series of xylene before mounting with entellan. All stained sections were analyzed under a camera-attached light microscope (Zeiss Axiolab 5), and representative micrographs were captured.

Statistical analysis

The minimum sample size was determined with the power analysis with online statistical software of the Inonu University website (https://biostatapps.inonu.edu.tr). Homogenity of the data was calculated by Levene's Test, and normality by Shapiro-Wilk Test. A one-way analysis of variance (ANOVA) was utilized using IBM SPSS Statistics for Windows (Version 24.0. Armonk, NY: IBM Corp.) to determine if the groups differed. If significant differences were detected, a post-hoc Tukey's Honestly Significant Difference (HSD) test was used to determine the differing groups. The data were presented as mean values ± SEM. The p values lower than 0.05 were considered statistically significant.

Results

Passive avoidance test

The PA test protocol started with the acquisition trial, followed by the retention trial (24 hours later) with no drug applications. In the acquisition trial, there were no differences in the transfer latencies of the mice ($p>0.05$, Figure 1).

In the retention trial of the PA test, transfer latencies of the meperidine and fentanyl-applied groups (MEP and FEN) were lower than the vehicle-treated group (VEH) ($p<0.01$, $p<0.05$, respectively). The latencies were higher in the meperidine-ketamine combination applied group (MK) compared with the MEP group ($p<0.05$, Figure 2).

Hematoxylin and eosin results

Histological structure and cellular organization were similar in the VEH group, with distinguishability of substantia grisea from substantia alba depending on the existence of cerebral neurons. The cerebral cortex in this group was covered with pia mater and contained vessels with various diameters, neurons, and glial cells. The hippocampus

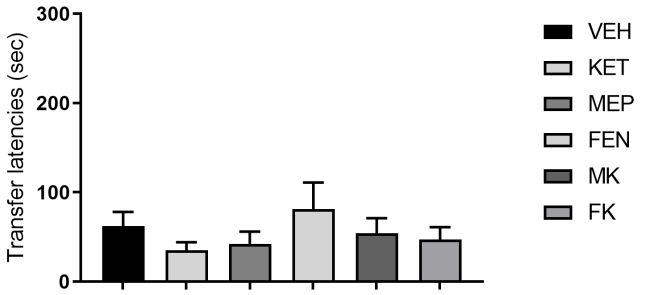

Figure 1. PA test acquisition trial, step-through latencies. VEH: vehicle, KET: ketamine (5 mg/kg), MEP: meperidine (10 mg/kg), FEN: fentanyl (0.3 mg/kg), MK: meperidine-ketamine, FK: fentanyl-ketamine. Each column represents the mean ± SEM of 7-8 mice. A one-way ANOVA was used for statistical analysis.

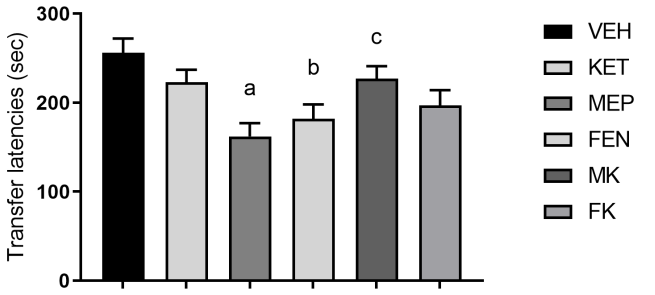

Figure 2. PA test retention trial, step-through latencies. VEH: vehicle, KET: ketamine (5 mg/kg), MEP: meperidine (10 mg/kg), FEN: fentanyl (0.3 mg/kg), MK: meperidine-ketamine, FK: fentanyl-ketamine. ap<0.01 vs. the VEH group, bp<0.05 vs. the VEH group, cp<0.05 vs. the MEP group. Each column represents the mean ± SEM of 7-8 mice. A one-way ANOVA followed by a post hoc Tukey's HSD test was used for statistical analysis.

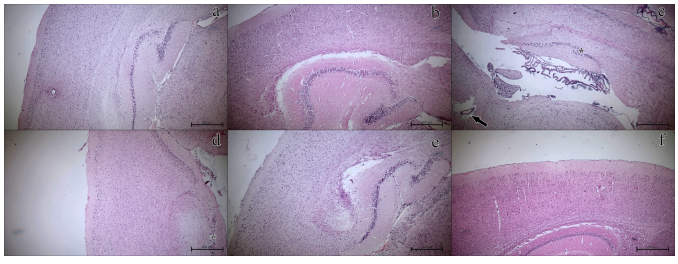

Figure 3. Representative micrographs of the cerebrum of all groups. VEH (a), KET (b), MEP (1c), FEN (d), MK (e), FK (f). Perivascular edema (thick arrow) and irregularity of the hippocampus (asterisk) were mainly observed in MEP and FEN groups. Staining: Hematoxylin and eosin. Bar: 200 µm.

is visible at the bottom of the cerebrum, and this structure is made of neurons, glial cells, and nerve fibers (Figure 3). The cerebral sections were examined with higher magnifications; the neurons with pyknotic nuclei and edematous perivascular cavities were apparent in the MEP and FEN groups compared to the VEH and ketamine-applied (KET) groups. The morphological structure of the cerebral cortex in the MK and fentanyl-ketamine combination applied (FK) groups was similar to that of the VEH and KET groups (Figure 4). The hippocampus proper in the VEH group contained cornu ammonis and the dentate gyrus, which were distinguishable from each other. Hippocampal morphology in the KET group was similar to the VEH group. In the MEP and FEN groups, the irregularity in cornu ammonis of the hippocampus was apparent; neuronal pyknosis in this cornu of hippocampus was widespread; pyknotic nuclei caused increased perineural cavity and spongy-like cellular morphology; the hippocampus vessels were covered with dense edema. However, hippocampus morphology in the MK and FK groups was better, and morphology was comparable to the VEH and KET groups (Figure 5).

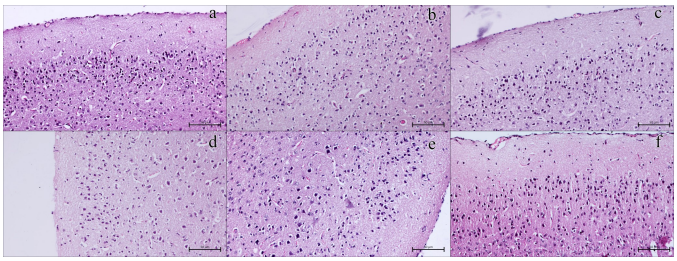

Figure 4. Micrographs of the cerebral cortex in VEH (a), KET (b), MEP (1c), FEN (d), MK (e), and FK (f) groups. Cerebral morphology in all groups was similar, besides neurons with pyknotic nuclei. Staining: Hematoxylin and eosin. Bar: 50 µm.

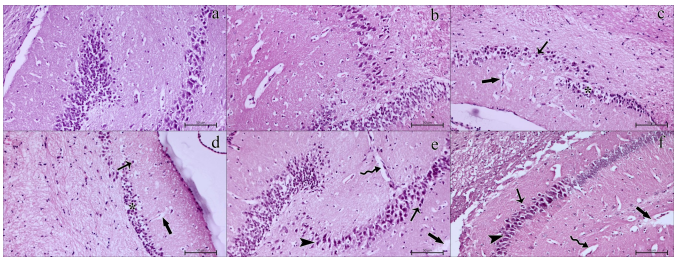

Figure 5. Micrographs of Hippocampus with higher magnification in VEH (a), KET (b), MEP (1c), FEN (d), MK (e), and FK (f) groups. Pyknotic neurons (arrow) in hippocampal portions of MEP and FEN groups. Perivascular cavity irregularity (thick arrow) in MEP and FEN groups due to accumulation of edema. The neurons in the MK and FK groups were normal (arrowhead) and similar to the VEH and KET groups. In MK and FK groups, regular vessels (curved arrow) were observed besides irregular perivascular cavity (thick arrow). Nerve tissue edema was slightly visible in some MK and FK group sections. Staining: Hematoxylin and eosin. Bar: 50 µm.

Discussion

Opioids are potent analgesic drugs commonly prescribed to alleviate severe pain. However, substantial evidence suggests a link between opioid use and memory impairments. Studies have indicated that chronic opioid use lead to cognitive deficits, affecting various aspects of memory [4,12]. As opioids are indispensable in pain management, there is a need to understand the mechanisms underlying the memory-impairing effects of opioids and to define protective measures against opioid-related cognitive decline.

Opioids exert their effects by binding primarily to the mu-opioid receptors. Mu receptors are widely distributed in regions associated with pain processing and areas involved in learning, memory, and cognition [1]. Prior studies have shown that opioid drugs may affect learning and memory processes [2,4]. One important parameter that affects memory impairments induced by opioids is the time interval and the dosing [13]. Although acute administrations were reported to cause memory impairments, high doses and continuous applications of opioids are responsible for cognitive decline [14,15].

Opioids influence the release of primarily dopamine and norepinephrine [16]. Disruptions in synaptic plasticity and alterations in neurotransmission may contribute to synaptic strength and connectivity adaptations, which may affect memory processes, including emotional memory functions [17]. A well-functioning emotional memory determines responses to situations and influences decision-making [18]. Opioids have been reported to interact with emotions and memory processing. The altered emotional state induced by opioids can impair emotional memory functions [19,20].

Fentanyl and meperidine are synthetic opioid drugs widely employed in pain management. While fentanyl is a pure opioid drug, meperidine also has anticholinergic effects, which makes it different from other opioids, especially when considering their memory-impairing effects. Ketamine, on the other hand, is an anesthetic drug that affects memory in a dose-dependent manner [21]. While higher doses and continuous applications cause memory impairments [22], some studies pointed out that low-dose ketamine may provide neuroprotection by increasing hippocampal neurogenesis [23], enhancing synaptogenesis [24], and synaptic connectivity [25].

This experiment employed a passive avoidance test to measure the emotional memory acquisition process. In the retrieval test, meperidine and fentanyl reduced transfer latencies of the mice, meaning emotional memory disruptions. Preclinical and clinical studies reported similar memory impairments related to these drugs [26-28]. The meperidine-ketamine combination applied group had higher transfer latencies than the meperidine-only applied group, indicating a reduction in the disruptive effects of meperidine by ketamine on emotional memory. A similar but insignificant increment was observed in the fentanyl-ketamine combination. These results indicate that ketamine may reduce cholinergic antagonism-related memory impairments but may not be as effective on opioid-related impairments.

Morphological studies in the literature have reported opioid-induced structural lesions in the hippocampus and cerebral cortex. Moreover, as a response to local cellular injury, upregulated glial fibrillary acidic protein expression, neuronal pyknosis in the hippocampus, and increased perineural cavities were reported [29,30]. When we compared our results with the literature, we found similarities, such as pyknotic nuclei in neurons and accumulation of edema at the perineural cavity. Although we observed perivascular edema accumulation, we did not observe any hemorrhage. This conflicting observation may result from different dose administrations in the literature.

However, most of the morphologically reported degenerations in literature are similar, and opioid exposure or addiction is related to neuronal degeneration. While there are some reported results related to fentanyl, we did not reach any study that examined the morphological effects of meperidine on the cerebral cortex or hippocampus; the data based on meperidine and neurodegeneration is quite limited. Opioids, as a significant drug family, are suspected agents that affect both functionality and morphology of the central nervous system.

Limitations

These findings only presented the effects of ketamine on memory deficits induced by two opioid drugs, meperidine and fentanyl. These drugs do not represent all opioids. More studies should be conducted to understand if ketamine is also effective against memory impairing effects of other opioids. Also, different doses of ketamine should be used to determine the best therapeutic range for this indication. Moreover, obtaining similar results may not be probable if an ischemia-reperfusion model was used to induce cognitive deficits. The literature indicates that the sensitivity of human and animal tissues is different. Accordingly, the results obtained in these studies may differ from those obtained with human samples and may be deceptive, which is the primary limitation of this study.

Conclusion

As opioids remain integral to pain management, concerns about cognitive decline last. Ketamine has a tremendous potential to fend against opioid-induced memory impairments and holds great promise. In this study, through low-dose ketamine combinations, opioid-induced neurodegenerative symptoms were eased, and memory impairment was alleviated against meperidine. The key finding of this study is the reduction of meperidine-induced cognitive deficits and neurodegeneration by low-dose ketamine. Meperidine may provide an alternative to fentanyl and may be combined with ketamine, mainly in hospital settings, instead of using fentanyl for most indications. More studies are necessary to unveil the mechanisms and the effects of altered application protocols of different opioids and drug combinations.

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Ethical approval

Approval for the study was received from Inonu University Animal Experiments Local Ethics Committee (Decision no: 2022/1-1).

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