Ketamine and its combinations with valproate and carbamazepine are ineffective against convulsions induced by atropine treatment and food intake in fasted mice

Neriman Gözüaçık 1, Ash Zengin Türkmen 2, Asiyu Nurten 2, Nurhan Enginar 1*

1 Department of Medical Pharmacology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey
2 Department of Physiology, Faculty of Medicine, Istanbul New Yüzüil University, Istanbul, Turkey

**Article history:** Received: Aug 3, 2018 Accepted: Oct 27, 2018

**Keywords:** Atropine, Carbamazepine, Convulsion, Fasting, Glutamate, Ketamine, Valproate

**Abstract**

**Objective(s):** Fasted rodents treated with antimuscarinics develop convulsions after refeeding. Food deprivation for 48 hr produces changes in [3H]glutamate binding suggesting glutamatergic contribution to the underlying mechanism of the seizures that are somewhat unresponsive to antiepileptics. Studies in animals and epileptic patients yielded considerable information regarding the anticonvulsant effect of the noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist ketamine. Thus, this study evaluated the efficacy of ketamine and its combinations with valproate and carbamazepine on convulsions in fasted animals.

**Materials and Methods:** Following 24 hr of fasting, mice were given saline, 5 or 10 mg/kg ketamine, 250 mg/kg sodium valproate, 24 mg/kg carbamazepine, 5 mg/kg ketamine, 5 mg/kg ketamine + sodium valproate, or 5 mg/kg ketamine + carbamazepine and then were treated with saline or 2.4 mg/kg atropine (5–9 mice per group). The animals were observed for the occurrence of convulsions after being allowed to eat ad libitum.

**Results:** Ketamine, valproate and carbamazepine pretreatments were ineffective in preventing the convulsions developed after atropine treatment and food intake in fasted animals. The incidence of convulsions was significantly higher in 5 and 10 mg/kg ketamine, carbamazepine, and carbamazepine + ketamine groups, but not in the valproate and valproate + ketamine treated animals.

**Conclusion:** In contrast to previous findings obtained with the NMDA antagonist dizocilpine (MK-801), ketamine lacks activity against convulsions developed after fasting. The drug does not enhance the efficacy of valproate and carbamazepine either. Using different doses of ketamine or other NMDA antagonists, further studies may better clarify the anticonvulsant effect of ketamine and/or role of glutamate in these seizures.

**Introduction**

Fasted mice and rats treated with muscarinic antagonists, scopolamine, atropine, biperiden, or pirenzepine develop convulsions soon after food intake (1, 2). Food deprivation itself, rather than its hypoglycemic consequence, seems to be critical in the occurrence of these seizures. Deprivation of food for 48 hr produced significant changes in the kinetics of (H)glutamate binding in the brain, which were partly antagonized by scopolamine treatment and food intake (3). Fasting increased expression of M1 muscarinic receptors in the frontal cortex and M2 muscarinic receptors in the hippocampus (2). Sight or smell of food, chewing, swallowing or the complete act of eating a meal may act as triggering factors (4). Dopaminergic D1 receptor antagonists chlorpromazine and haloperidol, glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 and alpha-2 adrenergic receptor agonists clonidine and tizanidine provided effective treatments. However, convulsions are somewhat unresponsive to most of the antiepileptic drugs (5, 6). Bearing some similarities in triggering factors and manifestations of the seizures and response to therapy in patients with eating epilepsy, convulsions in fasted animals may provide insight into the unknown underlying mechanism(s) of this rare form of reflex seizures (7, 8, 9).

The anesthetic drug ketamine is a glutamatergic noncompetitive NMDA receptor antagonist. It has potent analgesic and sedative effects (10). Animal studies have shown that ketamine exerts anticonvulsant effects in a variety of seizure models (11). The combinations of ketamine and conventional antiepileptics reduce seizure scores at non-effective doses (11, 12). Clinically, ketamine is used in the treatment of patients with status epilepticus refractory to benzodiazepines, barbiturates, and diphenylhydantoin (13). However, a proconvulsant effect for ketamine has also been documented (14, 15).

In view of the findings mentioned above, the present study was performed to evaluate the efficacy of ketamine and its combinations with valproate and carbamazepine on convulsions induced by antimuscarinic treatment and food intake after fasting. Atropine was used as the antimuscarinic drug (16).

**Materials and Methods**

**Animals**

Inbred male BALB/c mice weighing 25–35 g were
housed under standard laboratory conditions for at least 1 week prior to experimentation and were allowed free access to both food and water. All studies were approved by the Istanbul University Local Ethics Committee on Animal Experiments (2012/181) and were in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

**Drugs**

Atropine sulfate (Sigma, St. Louis, MO), ketamine (Ketalar, Pfizer, Turkey), and sodium valproate (Depakine, Sanofi, Turkey) were dissolved in saline, and carbamazepine (Novartis, Turkey) was suspended in 8.5% methylcellulose. Saline and drug solutions were given intraperitoneally (IP) in a volume of 4 ml/kg.

**Evaluation of the effects of ketamine and its combinations with antiepileptics on convulsions**

After being weighed, mice were moved to clean cages with fresh bedding and were deprived of food with free access to water. Following 24 hr of fasting, the animals were reweighed and given firstly saline, 5 or 10 mg/kg ketamine, 250 mg/kg sodium valproate, or 24 mg/kg carbamazepine pretreatments simultaneously with saline or 5 mg/kg ketamine injections. Ten minutes later, they were treated with saline or 2.4 mg/kg atropine. Group names and the treatments were shown in Table 1. Soon after saline or atropine administrations, mice in the control, atropine, ketamine 5 mg/kg, ketamine 10 mg/kg, ketamine 5 mg/kg+atropine, ketamine 10 mg/kg+atropine, valproate+atropine, valproate+ketamine+atropine, carbamazepine+atropine, and carbamazepine+ketamine+atropine groups were individually placed in wire mesh cages. Twenty minutes later, they were given food pellets and allowed to eat ad libitum. All mice were observed for 30 min for the incidence and onset of convulsions. Using a modified Racine’s scale (17), seizure activity was quantified by staging: (0) no difference; (1) freezing and gustatory movements; (2) forelimb clonus; (3) forelimb clonus with rearing; (4) forelimb clonus with rearing and falling down; and (5) generalized convulsions with rearing, falling down and jumping. A convulsive response was assessed as forelimb clonus with rearing. The onset of convulsions was defined as the time latency from starting to eat and appearance of first forelimb clonus with rearing. The incidence of convulsions was expressed as the percentage of animals displaying either stage 3, 4, or 5 activity in each group. The number of animals per group was 5–9.

Experiments were carried out between 09:00 and 15:00 hours in a temperature controlled (21±2 °C) quiet room. Observers were blind to the treatments.

**Statistical analysis**

The onset of convulsions data was evaluated using one-way analysis of variance (ANOVA). Fisher’s exact test \((n<20)\) was used for the evaluation of the frequency of the incidence of convulsions.

**Results**

After fasting for 24 hr, the body weights of the animals fell to approximately 84–87% of the starting body weights.

Table 2 shows that atropine treatment caused convulsions in fasted mice after food intake. The incidence of convulsions was statistically significant when compared with the control group \((P<0.01)\). Ketamine, valproate, and carbamazepine pretreatments were ineffective in preventing the development of convulsions. When compared with the control group, the incidence of convulsions was significantly higher in the ketamine 5 mg/kg+atropine \((P<0.01)\), ketamine 10 mg/kg+atropine \((P<0.001)\), carbamazepine+atropine \((P<0.01)\), and carbamazepine+ketamine+atropine \((P<0.01)\) groups. In contrast, the incidence of convulsions in the valproate+atropine and valproate+ketamine+atropine groups did not differ significantly from the control group \((P>0.05)\).

One-way ANOVA did not reveal main effect

---

**Table 1. Group names and treatments after 24 hr of fasting**

| Groups (n)            | First injections | Second injection |
|-----------------------|------------------|------------------|
| Control (8)           | saline + saline  | saline           |
| Atropine (9)          | saline + saline  | atropine         |
| Ketamine 5 mg/kg (5)  | saline + 5 mg/kg ketamine | saline          |
| Ketamine 10 mg/kg (5) | saline + 10 mg/kg ketamine | saline       |
| Ketamine 5 mg/kg + atropine (9) | saline + 5 mg/kg ketamine | atropine       |
| Ketamine 10 mg/kg + atropine (8) | saline + 10 mg/kg ketamine | atropine     |
| Valproate + atropine (8) | sodium valproate + saline | atropine |
| Valproate + ketamine + atropine (8) | sodium valproate + 5 mg/kg ketamine | atropine |
| Carbamazepine + atropine (9) | carbamazepine + saline | atropine |
| Carbamazepine + ketamine + atropine (9) | carbamazepine + 5 mg/kg ketamine | atropine |

\((n): number of animals\)
**Table 2. Effect of ketamine and its combinations with valproate or carbamazepine on atropine-induced convulsions in fasted mice after food intake**

| Groups (n) | Convulsions | Number of deaths c |
|------------|-------------|--------------------|
|            | Incidence (%) | Time of onset (min) [mean ± SE] h | |
| Control (8) | 0 | - | - |
| Atropine (9) | 67* | 5.3 ± 2.0 | 2 |
| Ketamine 5 mg/kg + atropine (9) | 78* | 4.7 ± 1.8 | 2 |
| Ketamine 10 mg/kg + atropine (9) | 88** | 4.6 ± 1.3 | 2 |
| Valproate + atropine (8) | 50 | 8.4 ± 4.5 | 2 |
| Valproate + ketamine + atropine (8) | 38 | 6.9 ± 2.2 | 2 |
| Carbamazepine + atropine (9) | 78* | 3.8 ± 1.3 | 2 |
| Carbamazepine + ketamine + atropine (9) | 78* | 5.0 ± 1.5 | 2 |

*Mice fasted for 24 hr were injected IP with saline, 5 or 10 mg/kg ketamine, 250 mg/kg sodium valproate, or 24 mg/kg carbamazepine pretreatments simultaneously with saline or 5 mg/kg ketamine 10 min prior to IP saline or atropine (2.4 mg/kg) treatments and were given free access to food 20 min later. ** Calculated from seizing animals. * Caused by generalized convulsions (stage 5).

**Table 3. Number of animals showing seizure stages in all groups**

| Groups (n) | Stage 0 | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 |
|------------|---------|---------|---------|---------|---------|---------|
| Control (8) | 8 | 0 | 0 | 0 | 0 | 0 |
| Atropine (9) | 0 | 0 | 3 | 2 | 1 | 3 |
| Ketamine 5 mg/kg + atropine (9) | 1 | 0 | 1 | 2 | 0 | 5 |
| Ketamine 10 mg/kg + atropine (8) | 1 | 1 | 0 | 3 | 0 | 4 |
| Valproate + atropine (8) | 0 | 2 | 2 | 3 | 0 | 0 |
| Valproate + ketamine + atropine (8) | 1 | 2 | 2 | 3 | 0 | 0 |
| Carbamazepine + atropine (9) | 0 | 1 | 1 | 4 | 0 | 3 |
| Carbamazepine + ketamine + atropine (9) | 0 | 0 | 2 | 2 | 0 | 5 |

(n): number of animals
by electrical stimulation (25) or by various chemical substances, such as NMDA (26), pilocarpine (27), lidocaine (28), 4-aminopyridine (22), bicuculline (29), pentylenetetrazole (30), and kainate (31). The doses used in the present study for ketamine, 5 and 10 mg/kg, were suggested to represent optimal doses for anticonvulsant activity. Nevertheless, seizures in fasted animals may require higher doses because of showing refractoriness to most antiepileptic treatments (5, 6). On the other hand, there are clinical and experimental observations for ketamine-induced convulsant or proconvulsant activity. Among those are the occurrence of seizures during anesthesia (32) or activation of epileptic foci (33) in patients and augmentation of pentylenetetrazole convulsions (34) or shortening of latency to sound-induced seizures (35) in animals. Ketamine did not produce any such activity at least in the dose range used in the present study.

Animal studies have shown that the combination of antiepileptic drugs with various agents could enhance the antiepileptic activity (36–38). In this respect, ketamine provided more effective treatments by potentiating the anticonvulsant effects of valproate and carbamazepine in seizures in rats and mice (12, 39). To investigate whether ketamine possesses similar efficacy in convulsions observed in fasted animal, the drug was combined with both antiepileptics. Carbamazepine at the dose of 24 mg/kg exerted prolongation of onset of convulsions, and sodium valproate at the dose of 250 mg/kg, partly suppressed the development of convulsions (5). Carbamazepine neither reduced the incidence nor delayed the onset of seizures. These findings are partly compatible with the previous findings. Ketamine coadministration did not provide an enhancement in carbamazepine’s activity. Lack of statistical significance in the incidence of convulsions in animals pretreated with valproate (50%) is consistent with the previous results (5, 40). Combination of valproate with ketamine produced a lower (38%), but also insignificant, incidence of convulsions compared with the atropine group (67%). The seizure stages in carbamazepine and valproate given animals were also unaffected by ketamine coadministration. Similar results were demonstrated when carbamazepine and lamotrigine were combined with the antidepressant amitriptyline (41).

**Conclusion**

Ketamine exhibited lack of anticonvulsant efficacy when given alone and produced no synergistic effect when given with carbamazepine or valproate in convulsions developed after atropine treatment and food intake in 24 hr fasted mice. As for the assessment of the role of glutamate in these convulsions, new studies with different doses of ketamine or other NMDA antagonists are required.

**Acknowledgment**

The results presented in this paper were from a student thesis. This work was supported by the Scientific Research Projects Coordination Unit of Istanbul University, Istanbul, Turkey (project number: 31620).

**Conflict of Interest**

The authors declare that there is no conflict of interest.

**References**

1. Enginar N, Nurten A. Seizures triggered by food intake in antimuscarinic-treated fasted animals: evaluation of the experimental findings in terms of similarities to eating-triggered epilepsy. Epilepsia 2010; 51(Suppl 3):80-84.
2. Saygın Bacanak M, Aydın B, Cabadak H, Nurten A, Gören MZ, Enginar N. Contribution of M1 and M2 muscarinic receptor subtypes to convulsions in fasted mice treated with scopolamine and given food. Behav Brain Res 2017; doi: 10.1016/j.bbr.2017.11.018.
3. Enginar N, Yamanıtkır P, Nurten A, Nurten R, Koyuncuoğlu H. Scopolamine-induced convulsions in fasted mice after food intake: determination of blood glucose levels, (3H)glutamate binding kinetics and antidopaminergic drug effects. Neuropearmacology 2003; 44:199-205.
4. Nurten A, Özerman B, Ozen I, Kara I. The role of solid fluid intake in antimuscarinic-induced convulsions in fasted mice. Epilepsy Behav 2009; 15:142-145.
5. Enginar N, Yamanıtkır P, Gören MZ, Ceylanı E, Ozer A, Akıncımez E. Scopolamine-induced convulsions in fasted mice after food intake: effects of glucose intake, antimuscarinic activity and anticonvulsant drugs. Neuropsychopharmacology 2005; 49:293-299.
6. Büger B, Türkmen AZ, Allahverdiyev O, Enginar N. Antimuscarinic-induced convulsions in fasted animals after food intake: evaluation of the effects of levetiracetam, topiramate and different doses of atropine. Naunyn-Schmiedebergs Arch Pharmacol 2016; 389:57-62.
7. Ahuja GK, Pauranik A, Behari M, Prasad K. Eating epilepsy. J Neurol 1988; 235:444-447.
8. Senanayake N. Reflex epilepsies: experience in Sri Lanka. Ceylon Med J 1994; 39:67-74.
9. Striano S, Coppola A, del Gaudio L, Striano P. Reflex seizures and reflex epilepsies: old models for understanding mechanisms of epileptogenesis. Epilepsy Res 2012; 100:1-11.
10. Mason KP, Michena E, DiNardo JA, Zurakowski D, Karian VE, Connor L, et al. Evolution of a protocol for ketamine-induced sedation as an alternative to general anesthesia for interventional radiologic procedures in pediatric patients. Radiology 2002; 225:457-465.
11. Chusemi M, Schachter SC. The NMDA receptor complex as a therapeutic target in epilepsy: a review. Epilepsy Behav 2011; 22:637-642.
12. Borowicz KK, Czućzwor SJ. Effects of etomidate, ketamine or propofol, and their combinations with conventional antiepileptic drugs on amygdala-kindled convulsions in rats. Neuropearmacology 2003; 45:315-324.
13. Prins H, Holthamp M. Ketamine successfully terminated malignant status epilepticus. Epilepsy Res 2008; 82:219-222.
14. Ferrer-Allado T, Brehner VL, Dymond A, Cozen H, Crandall P. Ketamine-induced electroconvulsive phenomena in the human limbic and thalamic regions. Anesthesiology 1973; 38:333-344.
15. Modica PA, Temelhoff R, White PF. Pro- and anticonvulsant effects of anesthetics [Part II]. Anesth Analg 1990; 70:433-444.
16. Lee W, Wolfe BB. Regulation of muscarinic receptor subtypes and their responsiveness in rat brain following chronic atropine administration. Mol Pharmacol 1989; 36:749-757.
17. Racine RJ. Modification of seizure activity by electrical modification. II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972; 32:281-294.
18. Parsons CG, Quack G, Bresink I, Baran L, Przegalinski E, Kostowski W, et al. Comparison of the potency, kinetics and voltage-dependency of a series of uncompetitive NMDA receptor antagonists in vitro anticonvulsive and motor
impairment activity in vivo. Neuropharmacology 1995; 34:1239-1258.
19. McDonald JF, Bartlett MC, Mody I, Pahapill P, Reynolds JN, Salter MW, et al. Actions of ketamine, phencyclidine and MK-801 on NMDA receptor currents in cultered mouse hippocampal neurons. J Physiol 1991; 432:483-508.
20. Parsons GG, Panchenko VA, Pichenko VO, Tsyndrenko AV, Krishtal OA. Comparative patch-clamp studies with freshly dissociated rat hippocampal and striatal neurons on the NMDA receptor antagonistic effects of amantadine and memantine. Eur J Neurosci 1996; 8:446-454.
21. Tricklebank MD, Singh L, Oles RJ, Preston J, Iversen SD. The behavioural effects of MK-801: a comparison with antagonists acting non-competitively and competitively at the NMDA receptor. Eur J Pharmacol 1989; 167:127-135.
22. Szakacz R, Weiczner R, Mihály A, Krisztin-Péva B, Zádor Z, Zádor E. Non-competitive NMDA antagonists moderate seizure-induced c-fos expression in the rat cerebral cortex. Brain Res Bull 2003; 59:485-493.
23. Durieux ME. Inhibition by ketamine of muscarinic acetylcholine receptor function. Anesth Analg 1995; 81:57-62.
24. Lorrain DS, Baccei CS, Bristow LJ, Anderson JJ, Varney MA. Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the prefrontal cortex: modulation by a group of II selective metabotropic glutamate receptor agonist LY379268. Neuroscience 2003; 117:697-706.
25. Manocha A, Sharma KK, Mediratta PK. Possible mechanism of anticonvulsant effect of ketamine in mice. Indian J Exp Biol 2001; 39:1002-1008.
26. Sharma AC, Thorat SN, Nayar U, Kulkarni SK. Dizocilpine, ketamine and ethanol reverse NMDA-induced EEG changes and convulsions in rats and mice. Indian J Physiol Pharmacol 1991; 35:111-116.
27. Freitas RM, Sousa FC, Viana GS, Fonteless MM. Effect of gabaaergic, glutamatergic, antipsychotic and antidepressant drugs on pilocarpine-induced seizures and status epilepticus. Neuroscience Lett 2006; 408:79-83.
28. Guler G, Erdogan G, Gologeli A, Akın A, Boyaci A. Ketamine reduces lidocaine-induced seizures in mice. Int J Neurosci 2005; 115:1239-1244.
29. Schneider PG, Rodrigues de Lorez Arnaiz G. Ketamine prevents seizures and reverses changes in muscarinic receptor induced by bicuculline in rats. Neurochem Inter 2013; 62:258-264.
30. Ghasemi M, Shafaroodi H, Nazarbeiki S, Meskar H, Heydarpour P, Ghasemi A, et al. Voltage-dependent calcium channel and NMDA receptor antagonists augment anticonvulsant effects of lithium chloride on pentylenetetrazole-induced clonic seizures in mice. Epilepsy Behav 2010; 18:171-178.
31. Twele F, Bankstahl M, Klein S, Römermann K, Löschner W. The AMPA receptor antagonist NBQX exerts antiseizure but not antiepileptogenic effects in the intrahippocampal kainate mouse model of mesial temporal lobe epilepsy. Neuropharmacology 2015; 95:234-242.
32. Kurdi MS, Sushima KS, Ranjana R, Kiran PB. Ketamine: a convulsant? Anesth Essays Res 2017; 11:272-273.
33. DeVore GR, McQueen JK, Woodbury DM. Ketamine hydrochloride and its effect on a chronic coxal epileptic cortical focus. Epilepsia 1976; 17:111-117.
34. Dashputra AV, Borkar AS, Hemmani TJ, Badwaik RT. Effect of ketamine on seizure activity and its interactions with antiepileptic drugs in rats. Int J Med Pharm Sci 2012; 3:1-8.
35. Bourn WM, Yang DJ, Davison JN. Effect of ketamine enantiomers on sound-induced convulsions in epilepsy prone rats. Pharmacol Res Commun 1983; 15:815-824.
36. Ghasemi M, Sharafeddine E, Rho JH, Shapkalski VN, Misrahi G, et al. Fosinopril and zofenopril, two angiotensin-converting enzyme (ACE) inhibitors, potentiate the anticonvulsant activity of antiepileptic drugs against audiogenic seizures in DBA/2 mice. Pharmacol Res 2012; 65:285-296.
37. Borowicz KK, Luszczki J, Czuczwar SJ. Effects of fluoxetine on the anticonvulsant action of valproate and ethosuximide in mouse model of myoclonic convulsions. Ann Agric Environ Med 2012; 19:487-490.
38. Borowicz KK, Piskorska B, Stepniak B, Czuczwar SJ. Effects of fluoxetine on the anticonvulsant action of valproate and ethosuximide in mouse model of myoclonic convulsions. Ann Agric Environ Med 2012; 19:487-490.