International Journal of Epilepsy 4 (2017) 144–149

Contents lists available at ScienceDirect

International Journal of Epilepsy

journal homepage: http://www.journals.elsevier.com/international-journal-of-epilepsy

Research paper

Amelioration of caffeine-induced seizures by modulators of sigma, *N*-methyl-D-Aspartate and ryanodine receptors in mice

Mojtaba Keshavarz[a,b,*], Seyyed Ahmadreza Hoseini[c], Samad Akbarzadeh[d]

[a] Department of Pharmacology, Bushehr University of Medical Sciences, Bushehr, Iran
[b] Shiraz Neuroscience Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[c] School of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran
[d] Department of Biochemistry, Bushehr University of Medical Sciences, Bushehr, Iran

ARTICLE INFO

Article history:
Received 6 November 2016
Accepted 25 September 2017
Available online 28 September 2017

Keywords:
Caffeine
Dantrolene
Diazepam
Ketamine
Opipramol

ABSTRACT

Objectives: The aim of this study was to evaluate the antiepileptic effects of opipramol, a sigma receptor agonist, diazepam, ketamine, an *N*-methyl-D-Aspartate (NMDA) receptor antagonist, and dantrolene, a ryanodine receptor antagonist, against caffeine-induced seizures in mice.
Methods: We used caffeine (1000 mg/kg) intraperitoneally for inducing clonic and tonic-clonic seizures in male albino Swiss strain of mice. We used opipramol in three different doses (10, 20 and 50 mg/kg), ketamine (50 mg/kg), dantrolene (40 mg/kg), opipramol (20 mg/kg) plus ketamine (50 mg/kg), opipramol (20 mg/kg) plus dantrolene (40 mg/kg), diazepam (5 mg/kg as a positive control) and the vehicle 30 min before injecting caffeine. We recorded the onset of clonic, tonic-clonic seizures and the time of death of animals after using caffeine.
Results: Animals treated with opipramol at a dose of 50 mg/kg or diazepam had a higher onset of clonic seizure compared with the vehicle-treated group. Dantrolene alone or with opipramol (20 mg/kg) increased the latency of clonic seizure compared with the control group. Opipramol (20 and 50 mg/kg), diazepam, ketamine alone or with opipramol, and dantrolene plus opipramol increased the latency of tonic-clonic seizures in mice. All the treatments except opipramol (10 mg/kg) and dantrolene alone increased the latency of death of animals.
Conclusion: Opipramol attenuated seizures produced by high doses of caffeine. Moreover, the activation of sigma receptors and inhibition of ryanodine receptors may produce synergistic effects against caffeine-induced seizures. Our study may imply that different mechanisms such as inhibition of gamma-aminobutyric acid-A receptors, activation of NMDA and ryanodine receptors may contribute to the caffeine-induced seizures.

1. Introduction

Caffeine, a methylxanthine derivative, is considered as the most widely used central nervous system (CNS) stimulants by man.[1] Caffeine is used in different foods and drinks like tea, coffee, and cola.[2] as well as over-the-counter medications such as headache preparations.[2] Furthermore, methylxanthines in the forms of theophylline and aminophylline, are widely used as medications to treat asthma[3] and apnea, especially in the newborns.[4]

In spite of widespread use of methylxanthines in the medicine and food industry, there are many reports about the serious side-effects of these agents, particularly in the toxic doses. A life-threatening seizure may be an important complication of methylxanthine therapy.[5] Methylxanthines are a trigger of the epileptic seizures in the patients without any history of epilepsy and a risk factor for the patients with underlying epilepsy.[6] More importantly, methylxanthine-induced seizures may be resistant to the conventional and new anti-epileptic drugs.[7,8]

The exact mechanism of methylxanthine-induced seizure is not completely understood. However, the inhibition of adenosine receptors and the activation of ryanodine receptors may be the main mechanisms responsible for the methylxanthine-induced seizure.[9] The ryanodine receptors activation increases the intracellular concentration of calcium in neurons, and this may

* Corresponding author at: Shiraz Neuroscience Research Center, Chamran Hospital, Chamran Boulevard, Shiraz, PO Box: 7194815644, Iran.
E-mail address: moj.ph60@yahoo.com (M. Keshavarz).

https://doi.org/10.1016/j.ijep.2017.09.003
2213-6320/

contribute to the convulsant activity of methylxanthines.[10] New generation antiepileptic drugs such as levetiracetam ameliorated peak height of intracellular calcium induced by caffeine.[11] Therefore, drugs that affect intracellular calcium may be useful for managing methylxanthine-induced seizures.

N-methyl-D-Aspartate (NMDA) and ryanodine receptors are actively involved in the intracellular calcium modulation. The ionic channels of NMDA receptors are highly permeable to calcium.[12] and raise intracellular calcium in neurons.[13] Moreover, ryanodine receptors are caffeine-sensitive calcium stores that mobilize calcium from intracellular pools.[10] Thus, the effects of NMDA or ryanodine receptors modulators on the intracellular calcium concentration may be useful for the control of caffeine-induced seizures.

Sigma receptors are chaperone proteins that are located on the sarcoplasmic reticulum.[14] These receptors have important roles in modulating NMDA receptors-mediated glutamate neurotransmission and intracellular calcium.[14,15] Some reports have implied that sigma receptors may be involved in the pathophysiology of epileptic seizure.[16] Sigma-1 receptor modulators like dextrorphan, carbetapentane, and pentazocine protected animals against kainic acid or maximal electroshock seizures.[17–19] Moreover, a specific sigma receptor agonist produced antiepileptic effects in the rat hippocampal slices.[20] Therefore, sigma receptor modulators may be the potential drugs for managing methylxanthine-induced seizure.

Opipramol is an antidepressant and anti-anxiety drug[21,22] with high affinity for the sigma receptors, particularly the sigma-1 subtype[22] and low affinity for the dopamine and NMDA receptors.[23,24] Opipramol exerted neuroprotective effects in the animal models of ischemia.[25] In our previous work this agent produced an anti-epileptic effect in the pentylenetetrazole (PTZ)-induced seizures.[26] However, there is no other report about the opipramol effects in the caffeine-induced seizure. Therefore, the aim of this study was to evaluate the antiepileptic effects of opipramol, a sigma receptor agonist, against caffeine-induced seizures in mice. We also aimed to show ketamine, a NMDA receptor antagonist, and dantrolene, a ryanodine receptor antagonist, effects against the caffeine-induced seizures in mice and their interaction with opipramol in this animal model.

2. Materials and methods

2.1. Chemicals

We bought opipramol and ketamine from Sigma (USA) and caffeine powder from Merck (USA). Diazepam and normal saline were procured from Daru Pakhsh Pharmaceutical Co., Iran. Opipramol, ketamine, dantrolene, diazepam and caffeine were dissolved in saline. We used all compounds by intraperitoneal (*i.p.*) injection 30 min before caffeine administration. All the compounds were used in a volume of 0.1 ml per 10 g of animal's body weight.

2.2. Animals and treatments

Male albino Swiss strain of mice was obtained from Razi Institute (Tehran, Iran). We kept animals in the Plexiglas cages (5 animals per cage) on a regular dark/light cycles (12 h/12 h), controlled temperature (22 ± 2 °C) and free access to food and water. Seventy-two mice were randomly allocated to the nine separate groups (n = 8). We used opipramol in three different doses (10, 20 and 50 mg/kg), ketamine (50 mg/kg), dantrolene (40 mg/kg), opipramol (20 mg/kg) plus ketamine (50 mg/kg), opipramol (20 mg/kg) plus dantrolene (40 mg/kg), diazepam (5 mg/kg as a positive control) and the vehicle 30 min before caffeine injection. The dose selection was mainly according to our previous studies on the dantrolene, opipramol, and ketamine effects against PTZ seizure.[26,27] The diagram in Fig. 1 shows drug using schedule. The experiment was approved by the local Animal Ethics Committee, which follows the European Communities Council to minimize the number and suffering of animals.

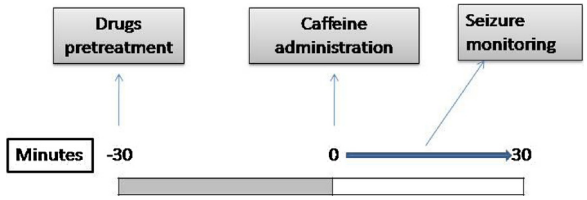

Fig. 1. The schedule of drug administration and seizure monitoring in mice. Mice were treated with drugs (opipramol (10, 20 and 50 mg/kg)), ketamine (50 mg/kg), dantrolene (40 mg/kg), opipramol (20 mg/kg) + ketamine (50 mg/kg), opipramol (20 mg/kg) + dantrolene (40 mg/kg), diazepam (5 mg/kg), and vehicle. All the treatments were used 30 min before the administration of caffeine (1000 mg/kg) and monitored for 30 min for the onset and occurrence of clonic and tonic-clonic seizures, and mortality.

2.3. Caffeine-induced seizure

We used caffeine (1000 mg/kg) to induce the clonic and tonic-clonic seizure in mice. After caffeine injection, mice were placed in the separate cages and watched for 30-min. According to the Łukawski et al. experiment,[28] we considered three seconds clonus of the whole animal body with loss of righting reflex as the clonic seizure. Generalized clonus of animal body with the extension of both forelimb and hindlimb was defined as the generalized tonic-clonic seizure. We recorded the latency of caffeine-induced seizures and death as the onset of clonic and generalized tonic-clonic seizures and the time of death of animals after using caffeine.

2.4. Data analysis

We reported data as the mean ± standard error of the mean (SEM) for the recorded variables. We analyzed the variables with the Kruskal-Wallis test followed by the Mann-Whitney *U* test. The significant level was considered the *p*-value of <0.05. Statistical analysis was performed by the SPSS software version 18.

3. Results

3.1. Effects of different treatments on the onset of caffeine-induced clonic seizure

Animals treated with opipramol at a dose of 50 mg/kg ($\chi^2 = 0.00$, $p = 0.001$) or diazepam ($\chi^2 = 0.00$, $p = 0.001$) had a higher onset of clonic seizure compared with the vehicle-treated group. However, the onset of clonic seizure in the animals treated with opipramol at the doses of 10 and 20 mg/kg was not significantly different from the vehicle-treated group ($\chi^2 = 25.00$, $p = 0.46$; and, $46\chi^2 = 25.50$, $p = 0.49$, respectively). Dantrolene alone or with opipramol (20 mg/kg) increased the latency of clonic seizure compared with the control group ($\chi^2 = 13.50$, $p = 0.05$; and $\chi^2 = 6.00$, $p = 0.006$, respectively). Moreover, the latency of clonic seizure in the animals treated with opipramol + dantrolene was significantly higher than the opipramol- (20 mg/kg) or dantrolene-treated groups ($\chi^2 = 9.00$, $p = 0.02$; and $\chi^2 = 11.00$, $p = 0.03$, respectively). The onset of clonic seizure in the animals treated with ketamine alone or with opipramol (20 mg/kg) was not significantly different from the vehicle-treated group ($\chi^2 = 24.00$, $p = 0.40$; and

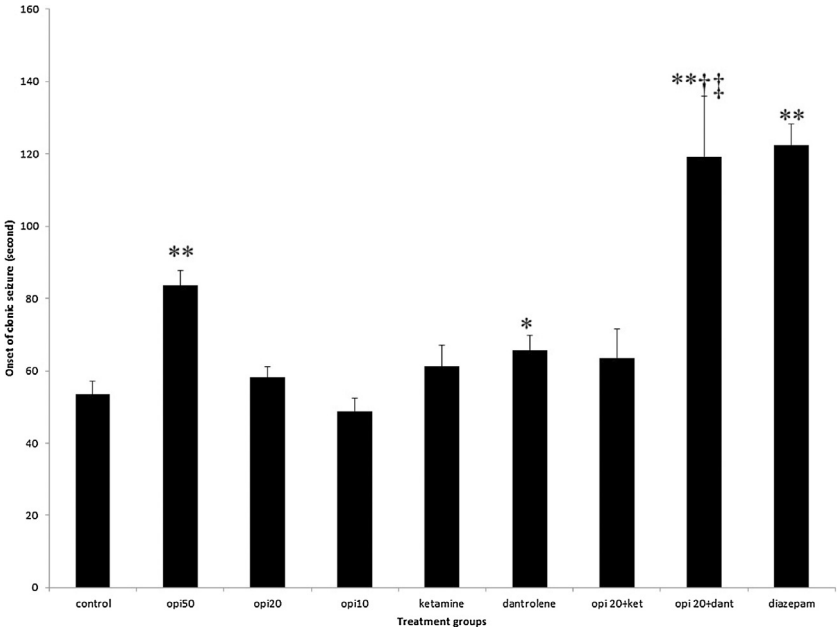

Fig. 2. The effects of different treatments on the onset of clonic seizure induced by caffeine in mice.
Drugs were administered interaperitoneally 30 min before the injection of caffeine. Data presented as mean ± standard error of mean and analyzed using Mann-Whitney *U* test. Each group consisted of 8 animals. * $p < 0.05$ and ** $p < 0.001$ compared with the vehicle-treated group, † compared with the opipramol (20 mg/kg) and ‡ $p < 0.05$ compared with dantrolene, opi: opipramol, ket: ketamine, dant: dantrolene.

$\chi^2 = 24.00$, $p = 0.40$, respectively). Moreover, the onset of clonic seizure in the animals treated with ketamine + opipramol (20 mg/kg) was not significantly different from ketamine ($\chi^2 = 29.50$, $p = 0.79$) or opipramol (20 mg/kg) ($\chi^2 = 27.00$, $p = 0.60$) groups. The effects of different treatments on the onset of clonic seizure induced by caffeine are summarized in Fig. 2. All animals in different groups including the diazepam group experienced tonic-clonic seizures.

3.2. Effects of different treatments on the onset of caffeine-induced tonic-clonic seizure

The animals treated with diazepam had a higher onset of tonic-clonic seizure compared with the control group ($\chi^2 = 0.00$, $p = 0.001$). The latency of caffeine-induced tonic-clonic seizure in the animals treated with opipramol at the doses of 20 and 50 mg/kg was higher than the vehicle-treated group ($\chi^2 = 10.00$, $p = 0.021$; and $\chi^2 = 13.00$, $p = 0.046$, respectively). However, opipramol at a dose of 10 mg/kg had no effect on the latency of caffeine-induced tonic-clonic seizure in mice ($\chi^2 = 24.500$, $p = 0.43$). Animals treated with ketamine or ketamine + opipramol (20 mg/kg) had a higher onset of tonic-clonic seizures compared with the control group ($\chi^2 = 12.00$, $p = 0.035$; and $U = 14.00$, $p = 0.06$, respectively). Furthermore, the latency of caffeine-induced tonic-clonic seizure in the animals treated with dantrolene + opipramol (20 mg/kg) was significantly higher than the vehicle-treated group ($\chi^2 = 4.00$, $p = 0.003$). Moreover, the onset of tonic-clonic seizure in the animals treated with dantrolene+ opipramol (20 mg/kg) was higher than the dantrolene ($\chi^2 = 9.50$, $p = 0.018$) or opipramol (20 mg/kg) ($\chi^2 = 10.00$, $p = 0.021$) groups. In contrast, the onset of tonic-clonic seizure in the animals treated with dantrolene was not significantly different from the vehicle-treated group ($\chi^2 = 17.00$, $p = 0.11$). The effects of different treatments on the onset of tonic-clonic seizure induced by the caffeine are summarized in Fig. 3. The 100% of animals in the all treatment groups including the diazepam group experienced tonic-clonic seizure. Moreover, there was no significant difference regarding the severity of seizure between different treatment groups ($p > 0.05$).

3.3. Effects of different treatments on the time of death of animals challenged with the convulsive doses of caffeine

All the animals, including diazepam-treated ones, died after the tonic-clonic convulsion induced by the higher doses of caffeine. However, diazepam ($\chi^2 = 0.00$, $p = 0.001$), opipramol (20 mg/kg) ($\chi^2 = 8.50$, $p = 0.013$), opipramol (50 mg/kg) ($\chi^2 = 9.00$, $p = 0.015$), ketamine ($\chi^2 = 8.00$, $p = 0.011$), ketamine + opipramol (20 mg/kg) ($\chi^2 = 6.50$, $p = 0.007$), and dantrolene + opipramol (20 mg/kg) ($\chi^2 = 1.00$, $p = 0.001$) increased the latency of death of animals challenged with high doses of caffeine. However, opipramol (10 mg/kg) or dantrolene alone had no effect on the time of death of animals challenged with high doses of caffeine. Fig. 4 shows the effects of different treatment on the latency of death of animals challenged with high doses of caffeine.

4. Discussion

Our study showed that opipramol, a sigma receptor agonist, attenuated the caffeine-induced seizures. There is no other study about the effect of sigma receptor agonists against methylxanthine-induced seizures. However, our previous study revealed that opipramol may be effective for the PTZ-induced seizure management.[26] We showed that opipramol increased the onset of PTZ-induced clonic and tonic-clonic seizures in mice.[26] Moreover, there are some reports about the anticonvulsant activity of sigma receptor modulators in different models of epilepsy. Sigma receptor agonists like carbetapentane, morphinan derivatives, and pentazocine attenuated seizures induced by different convulsants.[17–19] SKF83959, a selective sigma-1 receptor modulator, also ameliorated seizures induced by different convulsants such as maximal electroshock, PTZ, and kainic acid.[29] Therefore, sigma receptor agonists may be effective against different animal

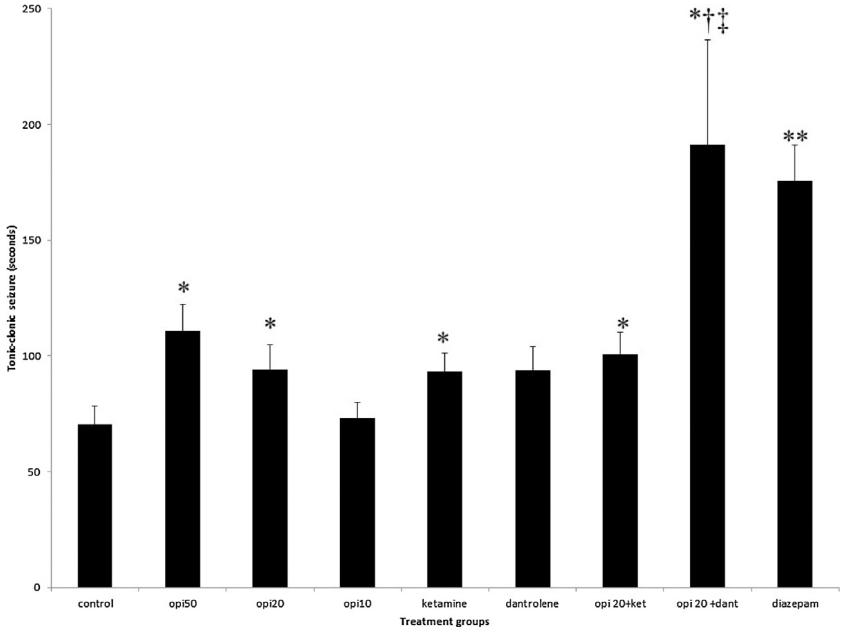

Fig. 3. The effects of different treatments on the onset of tonic-clonic seizure induced by caffeine in mice.
Drugs were administered interaperitoneally 30 min before the injection of caffeine. Data presented as mean ± standard error of mean and analyzed using Mann-Whitney *U* test. Each group consisted of 8 animals. * $p < 0.05$ and ** $p < 0.001$ compared with the vehicle-treated group, † $p < 0.05$ compared with opipramol (20 mg/kg), ‡ $p < 0.05$ compared with dantrolene, opi: opipramol, ket: ketamine, dant: dantrolene.

models of epilepsy and can be considered for the epileptic seizure treatment.

The exact mechanism of action of opipramol against caffeine-induced seizure is not completely clear. However, the intracellular calcium regulation via sigma-1 receptors may be an important target for the anticonvulsant activity of opipramol. Sigma receptors have been shown to block both voltage-gated calcium channels and ionotropic glutamate receptors.[30,31] On the other hand, caffeine-induced intracellular calcium release suppressed post-synaptic $GABA_A$ currents[32] and produced seizure.[33] Therefore, decreasing intracellular calcium by opipramol may suppress the inhibitory effects of caffeine on the $GABA_A$ receptors and increase the threshold for the caffeine-induced seizures.

Our study showed that different classes of drugs affect different seizure types induced by caffeine. In accordance, ketamine, a noncompetitive NMDA antagonist, suppressed caffeine-induced

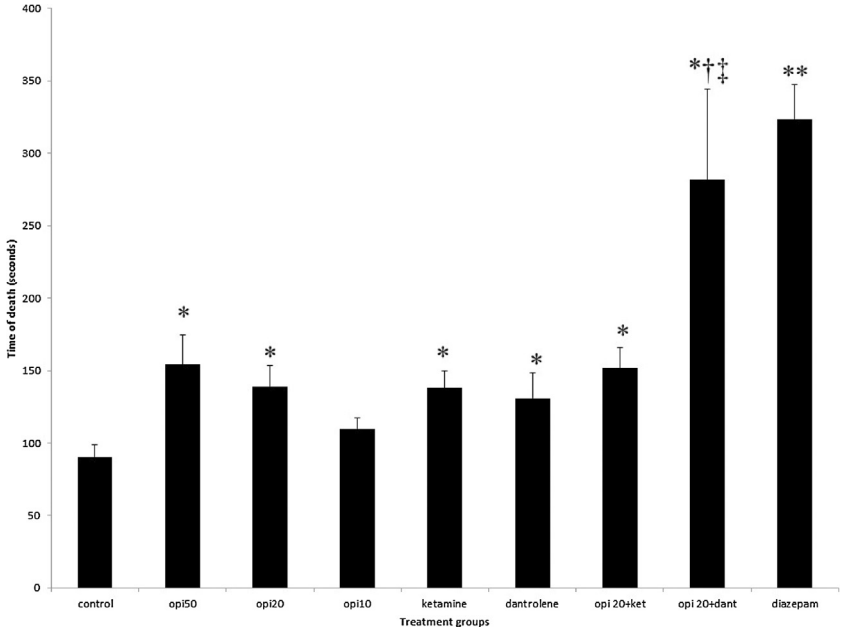

Fig. 4. The effects of different treatments on the time of death of animals challenged with caffeine.
Drugs were administered interaperitoneally 30 min before the injection of caffeine in mice. Data are presented as mean ± standard error of mean and analyzed using Mann-Whitney *U* test. Each group consisted of 8 animals. * $p < 0.05$ and ** $p < 0.001$ compared with the vehicle-treated group, † $p < 0.05$ compared with opipramol (20 mg/kg), ‡ $p < 0.05$ compared with dantrolene, opi: opipramol, ket: ketamine, dant: dantrolene.

tonic-clonic seizure while dantrolene, a selective ryanodine receptor antagonist, inhibited caffeine-induced clonic seizure. It is tentative to speculate that NMDA receptors may be involved in the tonic-clonic seizure. In this regard, non-convulsive doses of NMDA potentiated caffeine-induced seizures.[34] In addition, the activation of ryanodine receptors by caffeine may contribute to the clonic seizure. However, ketamine is not a selective inhibitor of NMDA receptors and at higher doses binds to other receptors such as opioid μ- receptors, sigma receptors,[35] and nicotinic acetylcholine receptors.[36] Taken together, it can be proposed that different pathways may be involved in various seizure types induced by high doses of caffeine.

The present study demonstrated that diazepam, a $GABA_A$ agonist, ameliorated both the clonic and tonic-clonic seizures induced by caffeine. In agreement with our study, Marangos et al.[37] showed that benzodiazepines suppressed caffeine-induced seizures. Therefore, it is likely that inhibiting $GABA_A$ receptors may be involved in both seizures produced by high doses of caffeine. However, in the present study, diazepam did not completely inhibit caffeine-induced seizure. Thus, other mechanisms such as the activation of NMDA and ryanodine receptors may contribute to the caffeine-induced seizures. Moreover, clinical data have shown that diazepam is not fully effective in the patients suffering from status epileptic-induced by the toxic doses of methylxanthines.[8] As mentioned, the toxic doses of caffeine may interact with different pathways other than $GABA_A$ receptors and blockade of one pathway may not be sufficient to inhibit methylxanthines-induced seizures.

In our study, using opipramol and dantrolene exerted higher effects against caffeine-induced seizures compared with the opipramol or dantrolene group alone. This implies that sigma receptor activation and ryanodine receptor inhibition may potentiate each other's effects against caffeine-induced seizures. Furthermore, different pathways for the calcium regulation may contribute to the caffeine-induced seizures. It has been demonstrated that deregulated calcium homeostasis and intracellular calcium elevation have provoked epileptic seizure.[38] Some studies have shown that ryanodine receptors have important roles in the intracellular calcium regulation and epileptic conditions.[39] Other studies have shown that caffeine-induced calcium release via ryanodine receptors may play important roles in the neuronal hyper-excitability and generation of seizure.[40] Moreover, dantrolene has suppressed caffeine-induced calcium release from the intracellular stores in the rat dorsal root ganglion neurons.[41] Therefore, dantrolene protective effects against caffeine-induced seizure may be related to the ryanodine receptors inhibition and reversing caffeine effects on the intracellular calcium. Moreover, sigma receptors modulate intracellular calcium by affecting both membrane channels and calcium mobilization from intracellular stores.[31] Therefore, potentiating effects of sigma receptors and ryanodine receptor antagonists on the intracellular calcium may augment the effects of these two classes of drugs against caffeine-induced seizures. However, the interaction of sigma receptors and ryanodine receptors has not been fully understood.

Other than the above-mentioned mechanisms, caffeine interaction with other systems may have a potential role in the convulsant effects of this substance. It has been suggested that the antagonistic effects of caffeine on the adenosine receptors may contribute to its pro-convulsant effects.[42]

The main limitation of our study may be the administration of non-specific receptor modulators like ketamine for studying caffeine-induced seizures. It is highly recommended to use specific receptor modulators in the future studies and to use the sigma receptor agonists in different seizure models like electroconvulsive shock or Kainic acid-induced seizure models.

5. Conclusion

Opipramol, a sigma receptor agonist, attenuated seizures produced by high doses of caffeine. Moreover, the activation of sigma receptors and the inhibition of ryanodine receptors may produce potentiating effects against caffeine-induced seizures. Drugs from different classes attenuated the caffeine-induced seizures in mice. This may imply that different mechanisms such as inhibition of $GABA_A$ receptors, activation of NMDA and ryanodine receptors, and increasing intracellular calcium may contribute to the caffeine-induced seizures. Moreover, the combination of drugs with different mechanisms of action may be more effective to control methylxanthine-induced seizures.

Conflict of interest

The authors have none to declare.

Author's contribution

Mojtaba Keshavarz: the design of the study, analysis and interpretation of data, drafting and revising the article and final approval of the manuscript.

Seyyed Ahmadreza Hoseini: acquisition of data, drafting the manuscript and final approval of the manuscript.

Samad Akbarzadeh: the design of the study, analysis of data, revising the manuscript and final approval of the manuscript.

Acknowledgments

We should acknowledge Dr. Iraj nabipour of providing the lab for performing this study and Mr. Adel Daneshi for his cooperation in the process of the study. We also appreciate Deputy of research of Bushehr University of Medical Sciences for the financial support of this study. We confirm that Deputy of research of Bushehr University of Medical Sciences had no role in the research conduction and preparation or submission of the manuscript.

References

1. James JE. *Understanding Caffeine: A Biobehavioral Analysis*. Michigan: Sage Publications, Inc; 1997:240.
2. Nehlig A, Daval J-L, Debry G. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res Rev*. 1992;17(2):139–170.
3. Tilley SL. Methylxanthines in asthma. In: Fredholm BB, ed. *Methylxanthines*. Springer; 2011:439–456.
4. Henderson-Smart DJ, Steer PA. Methylxanthine treatment for apnea in preterm infants. *Cochrane Database Syst Rev*. 2001;(3).
5. Boison D. Methylxanthines, seizures, and excitotoxicity. In: Fredholm BB, ed. *Methylxanthines*. Springer; 2011:251–266.
6. Delanty N, Vaughan CJ, French JA. Medical causes of seizures. *Lancet*. 1998;352 (9125):383–390.
7. Czuczwar SJ, Gasior M, Janusz W, Szczepanik B, Wlodarczyk D, Kleinrok Z. Influence of different methylxanthines on the anticonvulsant action of common antiepileptic drugs in mice. *Epilepsia*. 1990;31(3):318–323.
8. Yoshikawa H. First-line therapy for theophylline-associated seizures. *Acta Neurol Scand*. 2007;115:57–61.
9. Margineanu DG, Klitgaard H. Caffeine-induced epileptiform field potentials in rat hippocampal slices: a pharmacological characterization. *Neuropharmacology*. 2004;47(6):926–934.
10. McPherson PS, Kim YK, Valdivia H, et al. The brain ryanodine receptor: a caffeine-sensitive calcium release channel. *Neuron*. 1991;7(1):17–25.
11. Nagarkatti N, Deshpande LS, DeLorenzo RJ. Levetiracetam inhibits both ryanodine and IP3 receptor activated calcium induced calcium release in hippocampal neurons in culture. *Neurosci Lett*. 2008;436(3):289–293.
12. Ascher P, Nowak L. The role of divalent cations in the N-methyl-D-aspartate responses of mouse central neurones in culture. *J Physiol*. 1988;399:247.
13. Perkel DJ, Petrozzino JJ, Nicoll RA, Connor JA. The role of Ca2+ entry via synaptically activated NMDA receptors in the induction of long-term potentiation. *Neuron*. 1993;11(5):817–823.
14. Tsai S-Y, Hayashi T, Mori T. Su T-P: Sigma-1 receptor chaperones and diseases. *Cent Nerv Syst Agents Med Chem*. 2009;9(3):184–189.

15. Sha S, Qu WJ, Li L, et al. Sigma-1 receptor knockout impairs neurogenesis in dentate gyrus of adult hippocampus via down-regulation of NMDA receptors. *CNS Neurosci Ther*. 2013;19(9):705–713.
16. Nguyen L, Kaushal N, Robson MJ, Matsumoto RR. Sigma receptors as potential therapeutic targets for neuroprotection. *Eur J Pharmacol*. 2014;743(0):42–47.
17. Kim H-C, Jhoo W-K, Kim W-K, et al. Carbetapentane attenuates kainate-induced seizures via σ-1 receptor modulation. *Life Sci*. 2001;69(8):915–922.
18. Kim H-C, Shin CY, Seo DO, et al. New morphinan derivatives with negligible psychotropic effects attenuate convulsions induced by maximal electroshock in mice. *Life Sci*. 2003;72(16):1883–1895.
19. Khanna N, Khosla R, Kohli J. Opioid receptor mediated anticonvulsant effect of pentazocine. *Indian J Med Sci*. 1998;52(1):1–7.
20. Thurgur C, Church J. The anticonvulsant actions of sigma receptor ligands in the Mg2+-free model of epileptiform activity in rat hippocampal slices. *Br J Pharmacol*. 1998;124(5):917–929.
21. Möller H-J, Volz H-P, Reimann IW, Stoll K-D. Opipramol for the treatment of generalized anxiety disorder: a placebo-controlled trial including an alprazolam-treated group. *J Clin Psychopharmacol*. 2001;21(1):59–65.
22. Müller W, Siebert B, Holoubek G, Gentsch C. Neuropharmacology of the anxiolytic drug opripramol, a sigma site ligand. *Pharmacopsychiatry*. 2004;37 (S 3):189–197.
23. Musacchio JM, Klein M, Paturzo JJ. Effects of dextromethorphan site ligands and allosteric modifiers on the binding of (+)-[3H] 3-(-3-hydroxyphenyl)-N-(1-propyl) piperidine. *Mol Pharmacol*. 1989;35(1):1–5.
24. Rao T, Cler J, Mick S, et al. Neurochemical characterization of dopaminergic effects of opipramol, a potent sigma receptor ligand, in vivo. *Neuropharmacology*. 1990;29(12):1191–1197.
25. Rao T, Cler J, Mick S, et al. Opipramol, a potent sigma ligand, is an anti-ischemic agent: neurochemical evidence for an interaction with the N-methyl-D-aspartate receptor complex in vivo by cerebellar cGMP measurements. *Neuropharmacology*. 1990;29(12):1199–1204.
26. Keshavarz M, Yekzaman B. The evaluation of Opipramol, a sigma receptor agonist, effects against pentylenetetrazole-induced seizure in mice. *IJMS*. 2017; [in press].
27. Keshavarz M, Foutohi M, Rasti A. Dantrolen, a selective ryanodine receptor antagonist, protects against pentylenetetrazole-induced seizure in mice. *Acta Med Iran*. 2016;4(9):555–561.
28. Łukawski K, Czuczwar SJ. Effect of ACE inhibitors and AT1 receptor antagonists on pentylenetetrazole-induced convulsions in mice. *Neurol Sci*. 2015;36 (5):779–781.
29. Guo L, Chen Y, Zhao R, et al. Allosteric modulation of sigma-1 receptors elicits anti-seizure activities. *Br J Pharmacol*. 2015;172(16):4052–4065.
30. Zhang H, Cuevas J. Sigma receptors inhibit high-voltage-activated calcium channels in rat sympathetic and parasympathetic neurons. *J Neurophysiol*. 2002;87(6):2867–2879.
31. Monnet FP. Sigma-1 receptor as regulator of neuronal intracellular Ca2+: clinical and therapeutic relevance. *Biol Cell*. 2005;97(12):873–883.
32. Vigh J, Lasater EM. Intracellular calcium release resulting from mGluR1 receptor activation modulates GABAA currents in wide-field retinal amacrine cells: a study with caffeine. *Eur J Neurosci*. 2003;17(11):2237–2248.
33. Olsen RW, Avoli M. GABA and epileptogenesis. *Epilepsia*. 1997;38(4):399–407.
34. Inano S. Effects of agonists and antagonists of benzodiazepine, GABA and NMDA receptors, on caffeine-induced seizures in mice. *JPN J Psychopharmacol*. 1992;12(4):199–205.
35. Hustveit O, Maurset A, Øye I. Interaction of the chiral forms of ketamine with opioid, phencyclidine, σ and muscarinic receptors. *Pharmacol Toxicol*. 1995;77 (6):355–359.
36. Coates KM, Flood P. Ketamine and its preservative, benzethonium chloride, both inhibit human recombinant σ7 and σ4β2 neuronal nicotinic acetylcholine receptors in Xenopus oocytes. *Br J Pharmacol*. 2001;134(4):871–879.
37. Marangos P, Martino A, Paul S, Skolnick P. The benzodiazepines and inosine antagonize caffeine-induced seizures. *Psychopharmacology (Berl)*. 1981;72 (3):269–273.
38. DeLorenzo RJ, Sun DA, Deshpande LS. Cellular mechanisms underlying acquired epilepsy: the calcium hypothesis of the induction and maintainance of epilepsy. *Pharmacol Ther*. 2005;105(3):229–266.
39. Pal S, Sun D, Limbrick D, Rafiq A, DeLorenzo RJ. Epileptogenesis induces long-term alterations in intracellular calcium release and sequestration mechanisms in the hippocampal neuronal culture model of epilepsy. *Cell Calcium*. 2001;30(4):285–296.
40. Verkhratsky A. Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. *Physiol Rev*. 2005;85(1):201–279.
41. Usachev Y, Shmigol A, Pronchuk N, Kostyuk P, Verkhratsky A. Caffeine-induced calcium release from internal stores in cultured rat sensory neurons. *Neuroscience*. 1993;57(3):845–859.
42. Johansson B, Georgiev V, Kuosmanen T, Fredholm BB. Long-term treatment with some methylxanthines decreases the susceptibility to bicuculline-and pentylenetetrazol-induced seizures in mice. Relationship to c-ios expression and receptor binding. *Eur J Neurosci*. 1996;8(12):2447–2458.